CHROMOSOMAL ANALYSES OF THE GENETIC FACTORS FOR RESISTANCE TO DDT IN TWO RESISTANT LINES OF DROSOPHILA MELANOGASTER[1]

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THE data which had accumulated over a period of more than four years of experimenting with the problem of resistance to DDT in populations of *D. melanogaster* all pointed to the conclusion that resistance was inherited not as a single gene but as a polygenic complex. Numerous resistant lines had been produced by selection. In some the mean tolerance had been increased more than twentyfold. But response to selection was always slow and it was as rapid at a relatively low level of selective intensity when only the most susceptible half of the mortality distribution was eliminated, as it was when selection was more intense and only the five or ten percent of most resistant individuals were allowed to survive. Different strains responded differently to selection. Different parallel lines stemming from the same strain and selected in the same way did not respond identically. In some crosses between such lines it was quite clear that dissimilar factors for resistance were segregating in the F_2 (KING 1954, 1955a, b).

This evidence for a polygenic system as the basis of resistance was strong but it was mostly indirect and inferential. It therefore seemed advisable to try for more direct evidence by measuring separately the mean tolerance of a series of groups of flies, the flies in each group carrying one of the possible combinations of resistant and nonresistant chromosomes. The simplest way of doing this would have been to use chromosomes with dominant markers as the susceptible ones, but there were several reasons why it did not seem best to follow this procedure.

In the first place, flies carrying dominant markers, even if they are as viable as wild type flies (which they often are not), tend to behave differently. Curly-winged flies, for example, fly much less than wild type. Since all our measurements of resistance had been made by treating flies with an aerosol of DDT dissolved in tri-n-butyrin, it seemed very questionable to treat with an aerosol flies of widely different phenotypes having very different characteristics of behavior and to assume that the effective dosage would be the same. Secondly, earlier experiments had shown (KING 1955b) that in some crosses there was evidence of nonadditive interactions between genetic factors for resistance from different lines. Hence it seemed inadvisable to run tests on flies in which chromosomes from a resistant line and those from laboratory tester stocks were both

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present. In such a case we would have had no way of knowing whether the genetic material of the tester stock was interacting with the factors for resistance and if it was, in what direction and to what degree.

The material best suited to such an analysis appeared to be groups of wild type flies carrying the different possible combinations of resistant and susceptible chromosomes, the former drawn from a given resistant line and the latter from the control population from which the resistant line had been developed by selection. Such flies can be obtained by mating both susceptible and resistant flies to tester stocks carrying marked chromosomes and then making the appropriate series of matings between the resulting heterozygotes which carry marked chromosomes and known unmarked chromosomes.

MATERIALS AND METHODS

This type of analysis was carried through separately for two resistant lines—SyS-1002 and SyS-102. Both lines stemmed from a population of wild type flies descended from about two dozen individuals collected in a grocery store in Syosset, New York in July 1952. This Syosset population (designated by the symbol Sy) was maintained in the laboratory as a control, for the first year in mass cultures, from July 1953 on, in a population cage where the number of flies fluctuated between six and ten thousand. Samples from the population were tested continuously for resistance over a five year period and while there were fluctuations in LD_{50} through time, they were insignificant compared with the differences between the control and the two selected lines subjected to analysis.

SyS-1002 was set up in October, 1952. Selection was carried out by subjecting adult flies to an aerosol of DDT dissolved in tri-n-butyrin and using as the parents of the next generation several hundred flies which had survived a dose killing about 50 percent of those treated. A definite increase in resistance was apparent after about a dozen generations of selection. From this point resistance increased at a steady and more rapid rate until generation 40 when the LD_{50} had reached about 16 times that of the control. After F_{40} resistance continued to increase but at a somewhat slower rate. At F_{63} when flies were taken for the chromosome analysis the LD_{50} was about 20 times that of the control.

SyS-102 was set up in November, 1952 and selected at a higher level of intensity. The flies used as parents in this line had survived a dose of DDT killing approximately 95 percent of those treated. The number of pairs of parents varied from generation to generation from a minimum of four to a maximum of 20. At this intensity of selection there were too few offspring from the survivors to permit selection in every generation. These offspring were allowed to breed without exposure to DDT and in the following generation selection was repeated. Thus SyS-102 was selected only in alternate generations. There was no very pronounced response to selection until F_{34} when the LD_{50} reached four times that of the control. It remained at about this level until F_{50} when it suddenly jumped to 16 times. In subsequent generations it fluctuated around 20 times. Flies of F_{85} were used in carrying out the chromosome analysis.

For the purpose of making these analyses, two tester stocks were built up with dominant markers on the X, the second (II), and the third (III) chromosomes. In one of these the females carried attached-X chromosomes marked with yellow; in the other the X was Muller-5 carrying *sc*^{8} *w*^{a} *B*. In both stocks the II and the III were balanced lethals. One II was *SM-1* (KRAMER and LEWIS 1956) carrying *Cy al sp*, the other was the standard Plum (*Pm*) chromosome; one III was *Ubx*^{130} *e*^{8} (LEWIS 1952), the other Stubble bristle (*Sb*). Chromosome IV, which comprises a very small portion of the genetic material, was not followed in the analysis.

With two types of chromosomes—resistant and nonresistant—and three chromosome pairs, there are 27 different combinations possible in females. Two of these represent the resistant line and the control. Of the other 25, six have no heterozygous pairs of chromosomes and can be carried from generation to generation without producing recombined chromosomes partly of resistant and partly of nonresistant origin. The other 19 combinations are heterozygous in one or more pairs of chromosomes. By means of a mating scheme illustrated in the accompanying diagram, the six true-breeding combinations were produced. The 19 heterozygous groups were all obtained by making crosses using different combinations of the six true-breeding stocks, the resistant line itself or the control. For these 19 groups, only F_1 flies were tested so that no recombinant chromosomes were ever involved.

In the diagram (Figure 1) resistant wild type chromosomes are identified by a subscript "r" and nonresistant (susceptible) by a subscript "s". Since these

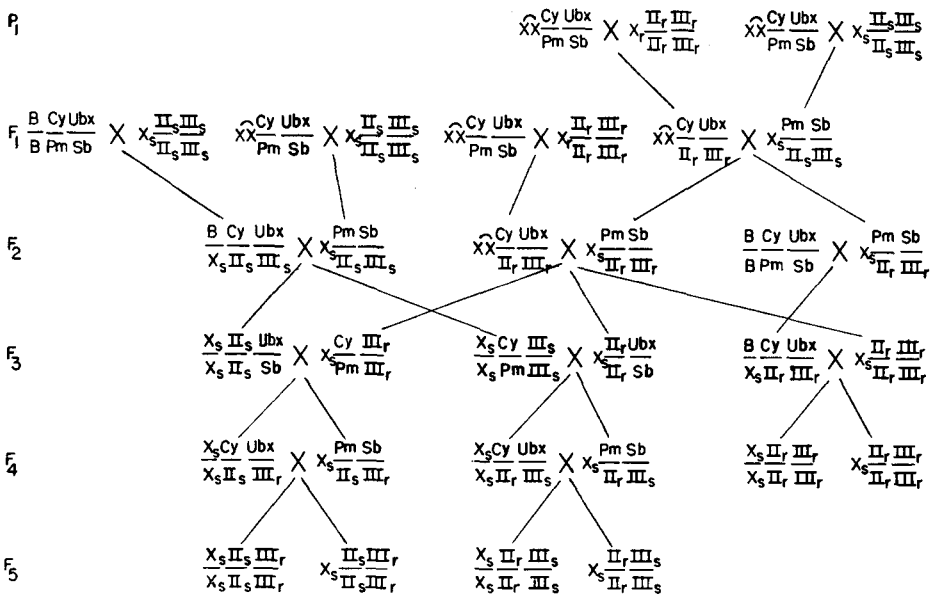

FIGURE 1.—Mating scheme used for obtaining different combinations of resistant and nonresistant wild type chromosomes.

symbols are cumbersome, we devised a simple code which will be used in the text. The nonresistant X, II, and III chromosomes are represented by 1, 2, and 3 respectively, and their resistant homologues by 4, 5, and 6. The control females are thus 11–22–33 and the resistant females 44–55–66. The females of the F_1 of a cross between these two lines would be 14–25–36. Each of the 27 combinations is for convenience identified by the karyotype of the female. The three true-breeding combinations shown as the result of the mating scheme in the diagram are, reading from left to right, 11–22–66, 11–55–33, and 11–55–66. The other three true-breeding combinations—44–22–66, 44–55–33, and 44–22–33—were obtained in a similar way, but the F_1 crosses were altered by using resistant males at the extreme left and females heterozygous for susceptible and males heterozygous for resistant chromosomes at the extreme right and by interchanging the positions of the two middle crosses.

It is well established that there are genetic differences between individual chromosomes isolated from any population. Consequently, it was desired to work with a sample array of different chromosomes for each pair drawn at random from the resistant line and from the control. This would give us, we hoped, a picture of what the chromosomes were doing in the randomly mating populations. An analysis made using a single chromosome from each pair from each population might give a very aberrant picture. To assure having such an array of different chromosomes, from 100 to 150 wild type males were mated to an approximately equal number of virgin females of the tester stocks in those P_1 and F_1 crosses where wild type males were used. These were divided among from 25 to 50 cultures, each culture having from three to four pairs of flies. When flies were collected from these cultures for use in crosses in the following generation, care was taken to collect approximately equal numbers of the phenotypes desired from each culture. This same procedure was followed throughout the mating program in order to insure the maintenance of a representative sample of different chromosomes.

In the course of the mating program the phenotypes to be used in subsequent crosses appeared in different ratios and for this reason the number of cultures per cross was varied. When a particular phenotype could be expected to appear with a low frequency, a larger number of cultures was set up. Usually 12 cultures were sufficient, but in some crosses from 20 to 30 were set up and, in two cases, 60. Each culture was set up with from two to four pairs of flies. The smallest total number of parents ever used was 18 females and 14 males divided among seven cultures. This was in the SyS-1002 series, the F_3 cross at the extreme right in the diagram.

As a result of these procedures we recovered in F_4 and F_5 wild type flies carrying almost no genetic material from the tester stocks. The *SM-1* and *Ubx* chromosomes contain complex multiple inversions and these were apparently effective in preventing recombination between tester and wild type chromosomes. No phenotypes indicating any such crossovers were ever observed. Throughout the mating program the only females ever used were those carrying these chromosomes. Since in the male there is no crossing over, males carrying *Pm* and *Sb*

chromosomes, which are less effective in preventing crossovers in the female, could be used. The Muller-5 chromosome also contains inversions but in the highly abnormal nucleus where the two large autosomes were also heterozygous for complex inversions, some crossovers between the X chromosomes occurred. These were manifested by males having w^a non-*B* eyes. Such males were discarded, but it was impossible to eliminate all recombinant X chromosomes because they could not be detected in heterozygous females. However, the total proportion of these recombinants was low. In the wild type flies which were tested for resistance, about three percent of the males had w^a eyes. The Y chromosomes also came from either the control (Sy) or the resistant line, but it was always from the source opposite to that of the X.

When the wild type flies were collected in F_4 and F_5 an effort was made again to get as large numbers as possible to insure having a reasonably good sample of wild type chromosomes. Table 1 summarizes the data on the number of females

TABLE 1

Number of wild type flies used to start stocks of the true-breeding combinations of resistant and nonresistant chromosomes and the number of cultures from which they were collected

Chromosome combination	Females	Males	Total	No. of cultures
		SyS–1002 Series		
11–22–66	274	227	501	57
11–55–33	370	278	448	59
11–55–66	25	9	34	7
44–22–66	131	117	248	24
44–55–33	113	99	212	24
44–22–33	30	23	53	15
		SyS–102 Series		
11–22–66	83	63	146	25
11–55–33	83	74	157	25
11–55–66	82	83	165	17
44–22–66	78	56	134	24
44–55–33	88	92	180	24
44–22–33	85	70	155	15

and males with which the stocks of the six true-breeding combinations of wild type flies were started. The number of cultures from which these were taken is also given. It can be seen that our minimum sample of chromosomes must have approximated two dozen and that in most cases we had considerably more.

The mating program was carried out first with SyS-1002. The P_1 crosses were made about the first of October, 1956 and the last F_5 offspring were collected about December 1. The testing of the combinations for resistance began December 17 and was finished on February 28, 1957. For SyS-102, the P_1 crosses were made about January 1, 1957 and the last F_5 offspring collected during the first week in March. The testing for resistance in the 102 series began on March 11 and was finished on May 2. Throughout the entire experiment the cultures were

kept at 25°C ±1° and the humidity in the laboratory was kept near 60 percent although there was no automatic control.

During the course of the testing program, we had to carry the six true-breeding stocks through four or five generations in order to have eggs of each stock available when they were needed. This was done by keeping the flies of each stock in a small population cage (4¼″ × 3½″ × 6″) and collecting eggs laid on food cups placed in the cages. The first generation of each stock was limited to the number of flies collected as shown in Table 1, but in all subsequent generations the number of flies in a cage was about 1000. Eggs from the control flies were obtained by placing sample cups in the control population cage. Eggs from the resistant lines were obtained from the populations which were still being subjected to selection. In neither line was there any substantial increase in resistance while the analysis was going on. In order to obtain eggs heterozygous for resistant and nonresistant chromosomes, pairs of cultures of the appropriate true-breeding, control or resistant lines were made up, 500 virgin females were collected from one set and 500 males from the other. These flies were then placed in a small population cage and the heterozygous eggs collected. To produce 11–25–36, for example, females from 11–55–66 were mated to control males (11–22–33).

Approximately 20,000 flies of each combination were raised in 100 culture bottles seeded with eggs collected in a population cage. Twenty-five bottles were seeded on each of four successive days. Fourteen days later the resulting flies were subjected in groups of 1000 to the aerosol of DDT. A series of time doses was selected for each combination to give a spread in mortality of from around ten percent to around 90 percent. The resulting dose-mortality figures were then plotted on a log probit scale and the LD_{50} calculated by the maximum likelihood method as described by Finney (1952). Thus a log LD_{50} for every one of the 27 chromosome combinations was obtained. For the control the figures were calculated from tests run on the control flies during the period when the other combinations were being tested. For the resistant lines the figures were those of the generation which had been used in the P_1 and F_1 crosses of the mating program.

Throughout the entire selection program male and female flies were treated together and mortality figures for the two sexes were not kept separately. For a number of technical reasons it was not practical to do otherwise. This same procedure was followed in the chromosome analysis. This means that all log-probit regression lines were based on composites of two mortality distributions with different means, one for males and one for females, for in all cases males as a group are less resistant than females. This has caused the variance of the mortality distribution and the error of the LD_{50} to be higher than they would have been if the two sexes had been counted separately. In the case of those chromosome combinations where the females were heterozygous for the X—all the 14's—there were two possible ways to make the cross. If females 44 were used, all males would have had resistant X chromosomes; if females 11 were used, all the males would have had nonresistant X chromosomes. All such crosses were made using females 44 because it was reasoned that this would reduce the difference between

the means of the mortality distributions of the sexes and thus reduce the error of th LD_{50}. Making the reciprocal cross would have increased the difference and the error.

RESULTS

Having obtained an LD_{50} value for every one of the 27 chromosome combinations, these data were subjected to a three-to-the-third power factorial analysis. For each chromosome pair there were three levels of genetic increment: no resistant chromosome, one resistant, and two resistant. Combining these three levels for the three pairs of chromosomes gave the 27 combinations. Early in the project it had been determined that a log-dose-probit plot of mortality data gave a better approximation to a normal distribution than any other simple transformation, and a large body of data from various crosses indicated that the genetic factors for resistance acted in a way which was additive on a logarithmic scale. Hence in carrying out the factorial analysis the logarithms of the LD_{50}'s in minutes of exposure were used.

Table 2 summarizes the results of such an analysis on the data for SyS-1002. Under Main Effects we get an answer to the question: Does the average X chromosome (or II or III) from SyS-1002 make a contribution to the resistant phenotype? The answers are unequivocal. All three chromosomes contribute something. Mean squares of these magnitudes could occur by chance alone in fewer than five experiments in ten thousand. Under First Order Interaction we discover whether, for example, a resistant X contributes equally in the presence or absence of a resistant II. Here, again, the answers are clear. There are no interactions between chromosome pairs taken two at a time. The mean squares are so low that values as large or larger should occur by chance half of the time. Finally, we find under Second Order Interactions that there is little if any interaction between the chromosome pairs taken three at a time. If any such interaction exists, it is less than four percent of the smallest of the main effects.

The breakdown of main effects into a linear and a quadratic component tells us what portion of the sum of squares for any one pair of chromosomes is attributable to a linear relationship between the mean LD_{50}'s of the three levels ($\bar{x}_0$, $\bar{x}_1$ & $\bar{x}_2$) and how much results from a quadratic component manifested by a significant deviation of $\bar{x}_1$ from the mean of $\bar{x}_0$ and $\bar{x}_2$. Here, again, the results are crystal clear. For all three chromosomes the linear component is far greater than the quadratic. For the latter, the mean squares are of an order which would be expected by chance more than a quarter of the time in the case of the X and the III and more than one tenth of the time in the case of the II. We can say, therefore, that for each pair of chromosomes, one resistant chromosome contributes half as much toward the resistant phenotype as two. Or, put in another way, there is no dominance.

In the lower portion of the table are given certain values of interest which can be deduced from the parameters we have measured. One of these is the contribution to the LD_{50} of a single chromosome of a given pair. These values differ, the

TABLE 2

Factorial analysis of the effects of chromosomes in producing resistance in SyS-1002

	Chromosome	Sum of squares	d.f.	Mean square	F ratio	P
Main effects	X	.50104	2	.25052	26.2	<.0005
	II	1.57859	2	.78929	82.7	<.0005
	III	.89363	2	.44681	46.8	<.0005
First order interaction	X–II	.01756	4	.00439	.5	>.50
	X–III	.02730	4	.00682	.7	>.50
	II–III	.02922	4	.00730	.8	>.50
Second order interaction	X–II–III	.07634	8	.00954	...	....
Breakdown of main effects						
Linear	X	.49427	1	.49427	51.8	<.0005
Quadratic	X	.00677	1	.00677	.7	>.25
Linear	II	1.56285	1	1.56285	163.8	<.0005
Quadratic	II	.01577	1	.01577	1.7	>.10
Linear	III	.88739	1	.88739	93.0	<.0005
Quadratic	III	.00624	1	.00624	.7	>.25

Chromosome			X	II	III
Mean for no resist. chr.	$\bar{x}_0$		.71279	.61213	.67841
Mean for one resist. chr.	$\bar{x}_1$		.91210	.85551	.86820
Mean for two resist. chr.	$\bar{x}_2$		1.04421	1.20145	1.12248
Grand mean	$\bar{x}$	.88970			
Contribution of one resistant chromosome to LD_{50}	$\frac{\bar{x}_2 - \bar{x}_0}{2}$		.16571±.05309	.29466±.05309	.22203±.05309
Expected LD_{50} of het. on linear scale	$\frac{\bar{x}_2 + \bar{x}_0}{2}$		.87850±.05309	.90679±.05309	.90045±.05309
Deviation of $\bar{x}_1$ from linear scale (Quadratic effect)	$\bar{x}_1 - \frac{\bar{x}_2 + \bar{x}_0}{2}$		+.03360±.09195	—.05128±.09195	—.03225±.09195

All errors show the 95 percent confidence limits: $\sigma \cdot t_{95}$

contribution of one X being least, that of one II greatest, and that of one III intermediate. The value for the X is significantly lower than that for the II at the 95 percent level. We can also compute the theoretical value of the heterozygote for each chromosome pair on the assumption that the relationship is entirely linear. By subtracting this from the corresponding $\bar{x}_1$ we obtain the deviation of the observed value from the theoretical line. In all three cases this deviation is smaller than its error. This is merely another way of showing that there is no quadratic component.

Knowing the contribution to the LD_{50} of a single chromosome of each pair, and assuming that the grand mean ($\bar{x}$) represents the value for the complete heterozygote (14–25–36), we can, by appropriate addition and subtraction, compute an expected LD_{50} for every one of the 27 combinations. In Table 3 these ex-

TABLE 3

Expected and observed log LD_{50}'s of combinations of chromosomes of SyS–1002 and Sy

Rank order	Combination	Expected log LD_{50}	Observed log LD_{50}	$-t\,\sigma$	$+t\,\sigma$
1	11–22–33	.20730	.31065	.23391	.35999
2	14–22–33	.37301	.35917	.27442	.41216
3	11–22–36	.42933	.43214	.32382	.45018
4	11–25–33	.50196	.58447	.53598	.62894
5	44–22–33	.53872	.61538	.57194	.66479
6	14–22–36	.59504	.62067	.57498	.66478
7	11–22–66	.65136	.70136	.65303	.74925
8	14–25–33	.66767	.58907	.53304	.64480
9	11–25–36	.72399	.65036	.57984	.71648
10	44–22–36	.76075	.71764	.67498	.76026
11	11–55–33	.79662	.77245	.69617	.83987
12	14–22–66	.81707	.85726	.81977	.89487
13	44–25–33	.83338	.81445	.74502	.89334
14	14–25–36	.88970	1.01413	.95859	1.07347
15	11–25–66	.94602	.76647	.72051	.81079
16	14–55–33	.96233	1.00609	.95103	1.06609
17	44–22–66	.98278	.89498	.79990	.98398
18	11–55–36	1.01865	.91703	.86002	.97678
19	44–25–36	1.05541	.88937	.84218	.93512
20	14–25–66	1.11173	1.08483	.98062	1.17958
21	44–55–33	1.12804	1.05402	.99476	1.10648
22	14–55–36	1.18436	1.15382	1.08545	1.21379
23	11–55–66	1.24068	1.28020	1.15484	1.38228
24	44–25–66	1.27744	1.30647	1.23896	1.37404
25	44–55–36	1.35007	1.41865	1.36182	1.47370
26	14–55–66	1.40639	1.52388	1.48615	1.55879
27	44–55–66	1.57210	1.68693	1.64410	1.73310

pected values are listed in ascending order along with the corresponding observed values and the 95 percent confidence limits of the latter. In Figure 2 these data are plotted. The regression equation of observed on expected is $y = 1.00000x \pm 0.00000$. What is more significant is that the variance of b is only 0.00243 and its standard error, 0.04930. The hypothesis that factors for resistance are additive on a logarithmic scale fits very closely with the experimental observations.

In Table 4 we have the results of exactly the same type of analysis on the data obtained from SyS-102. In this line also we have a highly significant main effect for each chromosome pair. There are no significant interactions between chromosome pairs taken two at a time and the second order interaction is extremely small in comparison with the main effects.

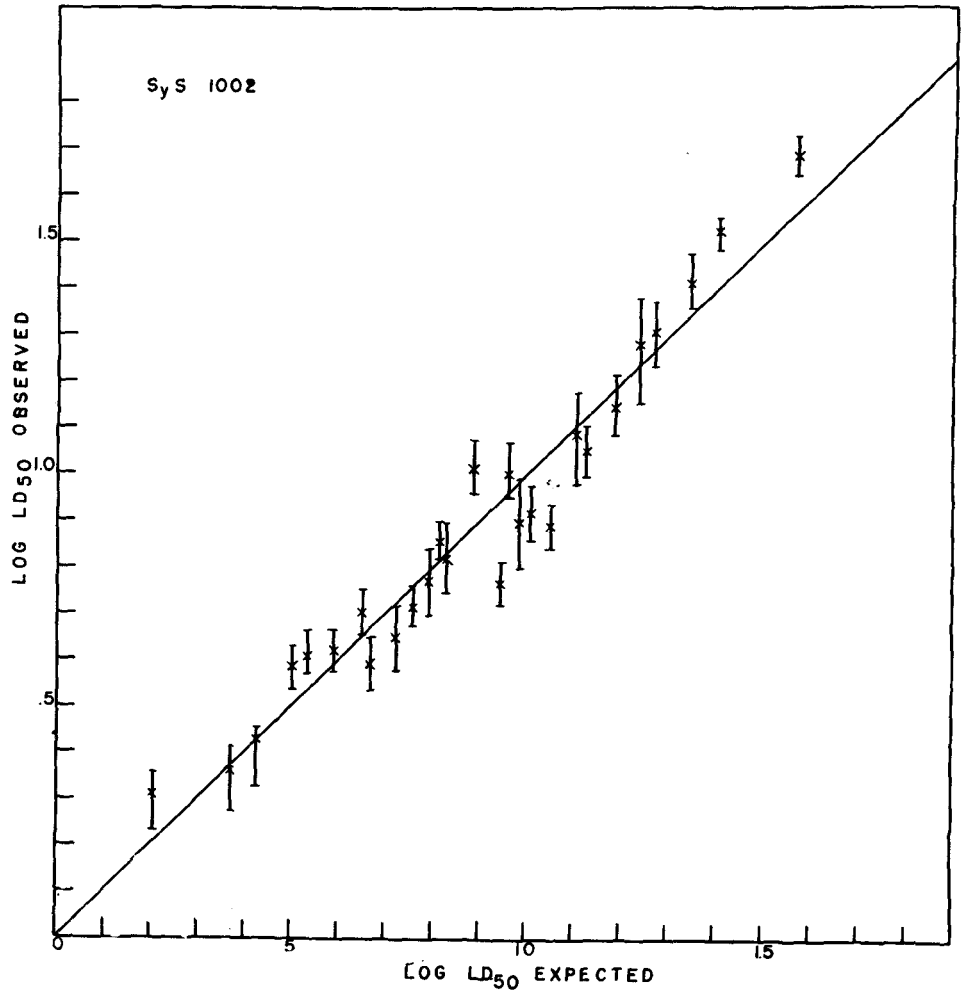

FIGURE 2.—Plot of observed log LD_{50}'s against expected values computed from contributions of individual chromosomes to LD_{50}. Crosses show LD_{50}'s; bars give 95 percent confidence intervals. Solid line is that of calculated regression equation.

Coming to the breakdown of main effects, we find one startling difference from what we found in SyS-1002. In SyS-102 there is a highly significant quadratic component for the X chromosome. There is no such component for either the II or the III. When we look at the deviation of the heterozygous X from the midpoint on the linear scale, we see again evidence of this quadratic component. This deviation is more than twice its error at the 95 percent level. The corresponding deviations for the II and III are both smaller than their errors. This means that in SyS-102 the resistant factors on the X chromosome are almost completely dominant.

The contributions of single chromosomes are unequal as they were in SyS-1002,

TABLE 4

Factorial analysis of the effects of chromosomes in producing resistance in SyS–102

	Chromosome	Sum of squares	d.f.	Mean square	F ratio	P
Main effects	X	1.95643	2	.97822	135.1	<.0005
	II	1.04275	2	.52138	72.0	<.0005
	III	.82015	2	.41008	56.6	<.0005
First order interaction	X–II	.04702	4	.01176	1.624	>.25
	X–III	.00308	4	.00077	.106	>.975
	II–III	.05101	4	.01275	1.761	>.10
Second order interaction	X–II–III	.05789	8	.00724		
Breakdown of main effects						
Linear	X	1.73012	1	1.73012	239.0	<.0005
Quadratic	X	.22512	1	.22512	31.9	<.0005
Linear	II	1.03813	1	1.03813	143.4	<.0005
Quadratic	II	.00463	1	.00463	.640	>.25
Linear	III	.79982	1	.79982	110.5	<.0005
Quadratic	III	.02031	1	.02031	2.805	>.10

Chromosome		X	II	III
Mean for no resist. chr.	$\bar{x}_0$	.71465	.83999	.85922
Mean for one resist. chr.	$\bar{x}_1$	1.21886	1.10795	1.12820
Mean for two resist. chr.	$\bar{x}_2$	1.33473	1.32030	1.28081
Grand mean	$\bar{x}$ 1.08941			
Contribution of one resistant chromosome to LD_{50}	$\frac{\bar{x}_2 - \bar{x}_0}{2}$	.31004±.04623	.24016±.04623	.21080±.04623
Expected LD_{50} of het. on linear scale	$\frac{\bar{x}_2 + \bar{x}_0}{2}$	1.02469±.04623	1.08015±.04623	1.07002±.04623
Deviation of $\bar{x}_1$ from linear scale (Quadratic effect)	$\bar{x}_1 - \frac{\bar{x}_2 + \bar{x}_0}{2}$	+.19417±.08010	+.02781±.08010	+.05819±.08010

All errors show the 95 percent confidence limits: $\sigma \cdot t_{95}$

but here the X makes the largest contribution and the III the smallest. The largest and smallest contributions are significantly different at the 95 percent level.

If, using the contributions of single chromosomes, we make the appropriate additions to and subtractions from the grand mean, we obtain a set of expected LD_{50}'s for all combinations and these together with the corresponding observed values and their 95 percent confidence limits are listed in ascending order in Table 5. A regression on the two sets of variables gives the equation: $y =$

TABLE 5

Expected and observed log LD_{50}'s of combinations of chromosomes of SyS–102 and Sy

Rank order	Combination	Expected log LD_{50}	Observed log LD_{50}	$-t\sigma$	$+t\sigma$
1	11–22–33	.32841	.38651	.33313	.42567
2	11–22–36	.53921	.58844	.53034	.64548
3	11–25–33	.56857	.52692	.48077	.56557
4	14–22–33	.63845	.67033	.63574	.70752
5	11–22–66	.75001	.61088	.55467	.66097
6	11–25–36	.77937	.69223	.65148	.73336
7	11–55–33	.80873	.50897	.47212	.54112
8	14–22–36	.84925	1.00377	.95368	1.05350
9	14–25–33	.87861	.98138	.94706	1.01526
10	44–22–33	.94849	.82912	.77994	.88786
11	11–25–66	.99017	1.01895	.96975	1.06759
12	11–55–36	1.01953	1.00597	.91093	1.10133
13	14–22–66	1.06005	1.15576	1.09419	1.22639
14	14–25–36	1.08941	1.23753	1.18816	1.28398
15	14–55–33	1.11877	1.28909	1.25425	1.32085
16	44–22–36	1.15929	1.09219	1.04277	1.14037
17	44–25–33	1.18865	1.16604	1.12876	1.20250
18	11–55–66	1.23033	1.09300	1.03813	1.14909
19	14–25–66	1.30021	1.42918	1.39445	1.46141
20	14–55–36	1.32957	1.51774	1.48152	1.55146
21	44–22–66	1.37009	1.22293	1.17511	1.26979
22	44–25–36	1.39945	1.28300	1.22190	1.34014
23	44–55–33	1.42881	1.37453	1.34029	1.40853
24	14–55–66	1.54037	1.68495	1.61827	1.78137
25	44–25–66	1.61025	1.63632	1.60390	1.66958
26	44–55–36	1.63961	1.73295	1.62234	1.95704
27	44–55–66	1.85041	1.67536	1.61057	1.73063

$0.99998x + 0.00002$, with a variance of b of 0.00459 and standard error of 0.06775. This variance is 1.89 times that which we obtained in the regression for SyS-1002. As an F-ratio this is not quite significant at the 95 percent level. Inspection of Table 5 suggests that a substantial portion of this variance arises from the 14 combinations which are consistently above their expected values. This is not surprising since the factorial analysis told us that the resistant X was dominant. If we correct each expected 14 value by adding to it the difference between $\bar{x}_1$ for the X chromosome and the grand mean (0.12944), we obtain a revised set of expected values corrected for dominance. These are listed in Table 6. In figure 3 the observed values are plotted against the corrected expected values. If we compute a regression on these figures we obtain: $y = 1.01368x - 0.05863$. We now have a negative intercept because we have increased some of the expected values without changing any of the observed, but the new line is essentially parallel to that for $y = x$ and the variance and standard error of b have decreased. These are now, respectively, 0.00227 and 0.04764. The value of

b is not significantly different from 1.0 and the variance is surprisingly close to that which we obtained for *b* in the case of SyS-1002, 0.00243. When we make allowance for the dominance, which is confined to the X, we find that the remaining factors for resistance in SyS-102 are additive on a logarithmic scale. The fact that the variances of the two *b*'s are so nearly equal and that the mean squares of the second order interactions of the two factorial analyses differ by a factor of only 1.3 indicates that experimental error must have been very nearly constant from one analysis to the other.

DISCUSSION

There has been a great deal of discussion of whether resistance to DDT is produced by a single factor or by a complex of factors, and published findings on this point have not all been in agreement. Part of the confusion seems to arise from the fact that two different questions are involved. One is: can we find a

TABLE 6

Expected and observed Log LD_{50}'s of combinations of chromosomes of SyS-102 and Sy. Correction made for dominance by adding .12944 to the expected value of every 14 combination

Rank order	Combination	Expected log LD_{50}	Observed log LD_{50}	$-t\,\sigma$	$+t\,\sigma$
1	11–22–33	.32841	.38651	.33313	.42567
2	11–22–36	.53921	.58844	.53034	.64548
3	11–25–33	.56857	.52692	.48077	.56557
4	11–22–66	.75001	.61088	.55467	.66097
5	14–22–33	.76789	.67033	.63574	.70752
6	11–25–36	.77937	.69223	.65148	.73336
7	11–55–33	.80873	.50897	.47212	.54112
8	44–22–33	.94849	.82912	.77994	.88786
9	14–22–36	.97869	1.00377	.95368	1.05350
10	11–25–66	.99017	1.01895	.96975	1.06759
11	14–25–33	1.00805	.98138	.94706	1.01526
12	11–55–36	1.01953	1.00597	.91093	1.10133
13	44–22–36	1.15929	1.09219	1.04277	1.14037
14	44–25–33	1.18865	1.16604	1.12876	1.20250
15	14–22–66	1.18949	1.15576	1.09419	1.22639
16	14–25–36	1.21885	1.23753	1.18816	1.28398
17	11–55–66	1.23033	1.09300	1.03813	1.14909
18	14–55–33	1.24821	1.28909	1.25425	1.32085
19	44–22–66	1.37009	1.22293	1.17511	1.26979
20	44–25–36	1.39945	1.28300	1.22190	1.34014
21	44–55–33	1.42881	1.37463	1.34029	1.40853
22	14–25–66	1.42965	1.42918	1.39445	1.46141
23	14–55–36	1.45901	1.51774	1.48152	1.55146
24	44–25–66	1.61025	1.63632	1.60390	1.66958
25	44–55–36	1.63961	1.73295	1.62234	1.95704
26	14–55–66	1.66981	1.68495	1.61827	1.78137
27	44–55–66	1.85041	1.67536	1.61057	1.73063

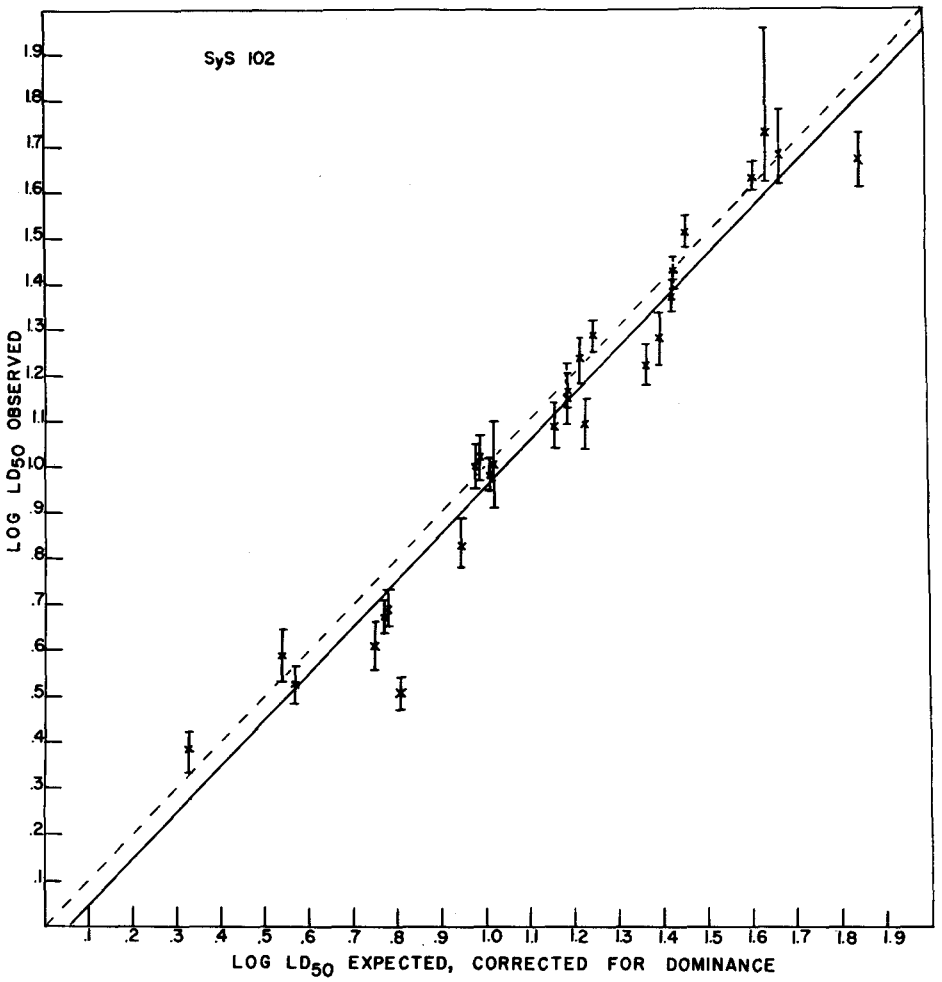

FIGURE 3.—Plot of observed LD_{50}'s against expected values computed from contributions of individual chromosomes to LD_{50} with corrections for the dominance of the X chromosome. Crosses show LD_{50}'s; bars give 95 percent confidence intervals. Solid line is that of calculated regression equation. Dotted line is that for $y = x$.

female and a male which differ in resistance and whose F_1 and F_2 offspring will be resistant or nonresistant in ratios which can be explained by regular Mendelian segregation of a single pair of alleles? The other is: are there sizable, randomly breeding populations differing in mean tolerance to DDT where this difference can be explained by a difference between the two populations in the frequencies of two alleles at a single locus?

The present analysis does not give a negative answer to the first question. We were careful to test a sample of numerous chromosomes in each of the three pairs. Our values for the contributions of individual chromosomes are therefore means for the samples of chromosomes tested. We cannot say that among the

chromosomes with which we worked a single set might not have been isolated which would have shown the major portion of resistance to segregate as a single gene. Nor can we give an unequivocal negative answer to the second question. What we can say is that we know of three populations which differ from each other with respect to several genetic factors. Each pair of chromosomes must have at least one locus affecting resistance. The X chromosome must have two such loci or three alleles at one, for it can be dominant or merely additive. There are factors which confer larval resistance without increasing adult resistance (KING 1955b). The fact that the relative potency of the three pairs of chromosomes differs between SyS-1002 and SyS-102 also argues for several factors on each. Neither of the two resistant populations tested owes its resistance to a single factor. And yet both stemmed from the same original stock and both were subjected to long and unrelenting selection. If the gene pool of the original stock had contained a gene which could produce high resistance with high efficiency, it is hard to see why it should not have become fixed as the basis for resistance in each subpopulation.

Most of the investigators who have worked on the genetics of resistance in Drosophila have concluded that they were dealing with polygenic systems (CROW 1957; BOCHNIG 1954; NACHTSHEIM and LÜERS 1954; OSHIMA 1954; SOKAL and HUNTER 1954). An outstanding exception to this consensus is afforded by OGAKI and TSUKAMOTO (1953) who found resistance attributable to a single gene which they were able to localize in a given region of chromosome II. However, the English summaries of their papers make it difficult to deduce all the details of the experimental procedures and furthermore, they were measuring larval tolerance, which seems to differ genetically from resistance in the adult.

It is in the work on resistance in pest insects, such as the house fly and the mosquito, where one finds many investigators espousing the single gene explanation. For some reason hard to comprehend many of these workers appear to take comfort and satisfaction in demonstrating that resistance is a simple Mendelian character. It is difficult to see any way in which such demonstrations contribute to the solution of problems of pest control. It is not necessary to observe simple Mendelian segregation of resistance to prove that this character is inherited. Anyone at all conversant with recent developments in the field of population genetics will not be surprised to find a complex and apparently disorderly array of graded phenotypes in any population. Such complexity and apparent disorder do not mean that the character in question is not under genetic control.

It is impossible here to review and evaluate the vast literature on the genetics of resistance in pest insects, but a brief discussion of one paper which provides very good evidence for a single gene difference can, we believe, establish that most of these papers have given an affirmative answer to only the first of the two questions posed in the first paragraph of this discussion. MAELZER and KIRK (1953) worked with a resistant Illinois strain of house flies in which about half the population survived a dose of 4μg/fly. These survivors they called "strong." Five single pair matings between strong Illinois flies and susceptible Canberra flies

gave, in two cases, all strong F_1 offspring and in three cases about half strong and half weak. It appeared that the strong Ilinois flies carried a single dominant gene and that two of the five had been homozygous and three heterozygous. The ratios in the F_2 of the two crosses involving the homozygotes were in substantial agreement with this hypothesis, but the F_2 ratios for the three crosses involving the heterozygotes deviated considerably from the expected, susceptible flies being in excess, and the three F_2 populations were statistically heterogeneous. MAELZER and KIRK suggest that this discrepancy arose because flies homozygous for the strong gene had reduced fertility. They were unable to maintain a homozygous strong stock.

The weak Illinois flies were still more resistant than the Canberra strain. Crosses between these two produced F_1's intermediate between the parents and F_2's not very different from the F_1's. The authors conclude that in the case of the weak Illinois flies "no simple genetic factor is involved whose effects are clear-cut." But in the F_2 there were a few flies (1.8 percent) which approached the Illinois strong flies in resistance.

There is no denying that these results demonstrate the presence of a single segregating factor producing high resistance. But the evidence also suggests strongly that this single factor may be a complex which under certain circumstances may remain intact through two generations, but which may also be broken up by recombination. This may be why the less resistant flies were consistently in excess in the Illinois strong × Canberra crosses and it may also explain why some F_2 flies from the Illinois weak × Canberra crosses were almost as resistant as the strong flies. In the first case the complex was broken up by recombination; in the second, something very nearly approaching it was synthesized. Surely MAELZER and KIRK's results do not give an affirmative answer to the second question and, as a matter of fact, they were careful to make no such claim.

SUMMARY

Five years of investigation of the inheritance of resistance to DDT in laboratory populations of *D. melanogaster* had led to the conclusion that resistance was produced by a polygenic complex. To confirm this, two resistant lines were subjected separately to chromosomal analysis. In the case of each line, an LD_{50} was obtained for flies of every one of the 27 possible combinations of the three large chromosome pairs: homozygous nonresistant, heterozygous and homozygous resistant for each pair. The nonresistant chromosomes were taken from the control stock from which the resistant lines had been developed by selection. The LD_{50}'s were then subjected to a factorial analysis. This showed that factors for resistance were located on each of the three chromosomes and that their apportionment among the three was not the same in the two lines. All factors were additive on a logarithmic scale except for some factors on the X chromosome in one line. The meaning of the results obtained for the problem of the inheritance of resistance in wild populations of pest insects is discussed.

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