CHROMOSOMAL ANALYSIS OF DDT-RESISTANCE IN A LONG-TERM SELECTED POPULATION OF *DROSOPHILA MELANOGASTER*[1]

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ABSTRACT

The genetic basis of DDT-resistance was studied in a population of *Drosophila melanogaster*. This population was unique in that it had been continually selected for DDT-resistance since 1952 and had achieved a very high level of resistance. The genetic basis of resistance was studied by means of a chromosomal analysis. Fifteen combinations of resistant and control chromosomes were tested using a time-based DDT test. The analysis of the data showed that resistance was multifactorial with each of the three major chromosomes involved. Dominant and recessive second and third chromosome effects were found to be much more important than those of the first chromosome, which had no detectable recessive effects. Second and third chromosome resistance genes showed incomplete dominance. The average dominance of the second chromosome was much less than that of the third chromosome. These large-scale differences between chromosomes' effects and average dominance may indicate that a small number of resistance genes are involved. Two significant interactions between chromosomes were found. Scaling difficulties make the interactions difficult to interpret without further data. It seems possible that positive interactions between resistance have been developed by the long-term directional selection in this population.

THIS paper reports the results of a chromosomal analysis performed to investigate the genetic basis of DDT-resistance in a highly resistant population of *Drosophila melanogaster*. Although studies on a number of other DDT-resistant populations have been reported, the population we utilized was unique in that it had been selected for a long period of time (23 years at the time of the analysis) and had achieved a very high level of resistance. Studies by MERRELL (1960) 16 years ago indicated that the $E.D._{50}$ of the resistant population used was more than 70 times that of the control. ($E.D._{50}$ is the effective dose of DDT required to knock down 50% of the test population. Flies that have been knocked down rarely, if ever, recover.) There are indications that the resistance has increased

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still further since that time. An unselected control population derived from the same starting population was available for use as a comparison.

The purpose of performing the chromosomal analysis was to investigate several aspects of the genetic basis of the observed DDT-resistance. How many genes are involved? What are their dominance relations? Do they interact? A complete answer to these questions must await further analysis, but these results permit a comparison with the results of a number of earlier studies of DDT-resistance in Drosophila (BOCHNIG 1954; CROW 1957a; KING 1954, 1956; KING and SOMME 1958; KIKKAWA 1958; OGAKI and TSUKAMOTO 1953; TSUKAMOTO and OGAKI 1953; OSHIMA 1958).

MATERIALS AND METHODS

The chromosomal analysis reported in this paper involved testing a series of genotypes derived from two populations, one highly resistant to DDT (91R) and the other a control never exposed to DDT (91C). Details of the origin, selection and maintenance of these populations are given in MERRELL and UNDERHILL 1956; MERRELL 1960, 1965; UNDERHILL and MERRELL 1966.

Briefly, the two populations were started from a common base population founded from a collection of several hundred *Drosophila melanogaster* caught in St. Paul, Minnesota, during September of 1952. The flies were captured, and the populations were maintained by one of us (DJM) until the tests reported here were made.

Once established, the two populations were maintained in population units consisting of two half-pint milk bottles joined by a piece of rubber radiator hose (REED and REED 1948). One of the half-pint slants of standard cornmeal, corn syrup, agar medium was changed, alternatively, every three weeks, which allowed the continuous maintenance of a population of several hundred flies.

Selection for DDT-resistance was practiced on 91R by placing a 1″ × 3″ piece of filter paper impregnated with DDT in each fresh food bottle. Progressive selection was achieved by putting increased amounts of DDT on the filter paper. The 91R population was initially started at .1 mg DDT. It has been maintained at 150 mg since 1968.

The populations have been maintained in a controlled temperature room. The temperature was maintained at 21 until 1970 when it was changed to 24°. All flies used in this experiment were grown and tested at 24° ± 0.5°. The relative humidity was controlled at 51 ± 1% during the tests.

The DDT test used was a time-based test. Batches of about 30 female flies were placed on DDT-coated petri dishes. Periodic observations of the number of flies no longer able to stand (knocked down) allowed the calculation of the average time before knockdown (Average Survival Time). Average Survival Time was calculated on the assumption that the flies knocked down during a time period succumbed at the midpoint of the interval.

Plates were set up near 8:00 A.M. each day of the six-day test period. Readings were made at regular two- or four-hour intervals. DDT plates were made by pipetting 1 ml of an acetone solution containing 100 mg of DDT onto the bottom of a standard 100 mm petri dish. The plates were "conditioned" prior to the tests by allowing five batches of 50 flies to remain on them for 24 hours. A total of 79 DDT plates was used. Clean petri dishes were used as controls for mortality not caused by DDT.

Test flies available for a day were assigned to the plates by a random process. For most of the strains, 19 replicate tests were run on 19 different plates over a six-day time period. On the second and fourth days a set of flies was run on clean plates to estimate mortality not caused by DDT.

The strains of flies tested had different combinations of 91C and 91R chromosomes. Chromosome combinations were manipulated using a system introduced by ROBERTSON and REEVE

(1955) and modified by LATTER (1973). A *Cy/Pm; TM3/D3* marker strain was used. The *Cy* chromosome has paracentric inversions in both arms. See LINDSLEY and GRELL (1968) for a description of the dominant markers and the *TM3* balancer chromosome.

The four generations of crosses used to prepare the 17 chromosomal combinations tested are shown in Figure 1. In both the first and second generations large samples of females from 91R

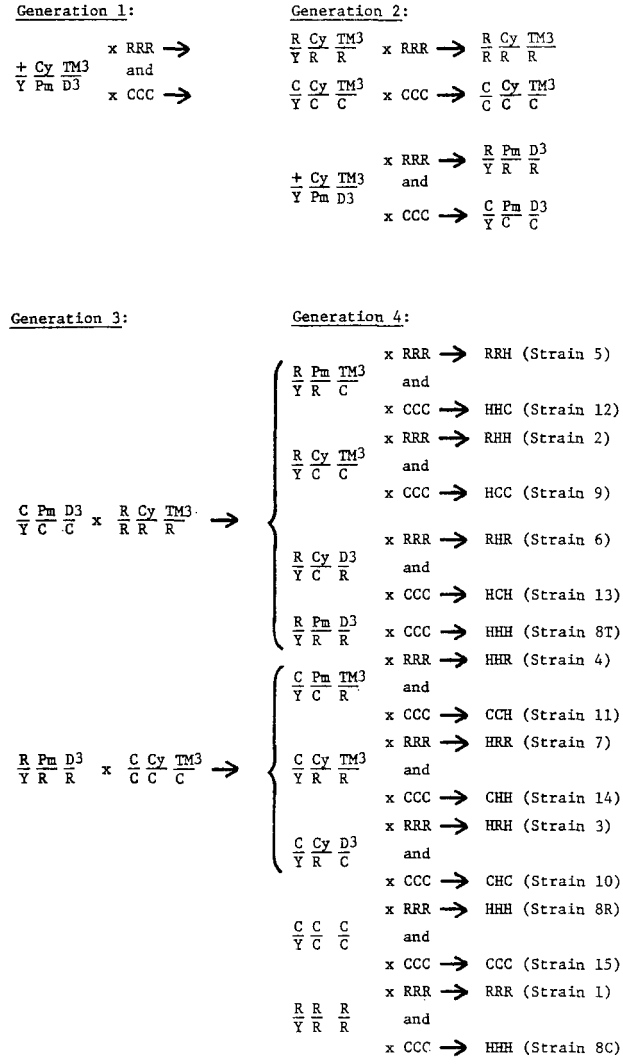

FIGURE 1.—Crossing procedure for the chromosomal analysis. This figure shows the four generations of crosses used to produce the 17 chromosomal combinations tested.

In this figure R and C refer to flies homozygous for a pair of chromosomes from strain 91R or 91C, respectively. The first (the *X*), second and third chromosomes of a genotype are denoted by their position, left to right. A (+) refers to a unspecified *X* chromosome from the marker strain. H means heterozygous with one chromosome from each parental population. Strains 8C, 8R and 8T are all HHH (F_1) flies prepared by three different methods as explained in MATERIALS AND METHODS.

and 91C were crossed to *Cy/Pm; TM3/D3* males. In the second generation *Cy;TM3* males were also backcrossed to 91R and 91C to produce the female parents for the third generation of crosses. These females were the only heterozygous females used in the procedure. Crossing over was supressed in them by the *Cy* and *TM3* chromosomes. In the fourth generation, 13 to 16 bottles of each cross were set up with 10 virgin females and 4 males as parents. Wild-type females from these bottles were tested. Test flies of each strain were collected daily, mixed, and aged for 3 days in freshly yeasted food bottles before testing.

Two genetic sources of error are possible in this chromosome analysis procedure. First, there might be genetic differences related to resistance segregating on the fourth chromosome. Second, double crossovers within the inversions might transfer genetic material from the inverted chromosome to the chromosomes being tested. Presumably, such transfers would make a greater difference when the inverted chromosome material was transferred to a resistant chromosome, as the inverted chomosome is more likely to resemble the control than the resistant chromosome. No experimental check was made on the possibility of an effect of the fourth chromosome, but its small size makes it an unlikely source of error.

The effect of double crossovers on the efficiency of the test procedure was investigated by tests of an additional genotype denoted 8T. To produce this genotype, *Pm; D3* males from the cross $\frac{C}{Y}\frac{Pm}{C}\frac{D3}{C}$ males $\times \frac{R}{R}\frac{Cy}{R}\frac{TM3}{R}$ females were crossed to virgin C females. The wild-type offspring of this cross should be identical to a heterozygous female, except that they have a reduced probability of an R fourth chromosome. Any drop in resistance of this genotype compared to the F_1 indicates the effect of unsuppressed crossing over within the inversions.

RESULTS

The basic unit for the analysis of the data was the logarithmically transformed Average Survival Time (AST). This statistic was calculated as:

$$\mathrm{AST} = \frac{1}{n}\sum_{i}^{R} \ln T_i \ ,$$

where T_i is the midpoint of the time interval in which the i^{th} fly died, and n is the total number of flies on a DDT plate.

The log transformation was performed because it most successfully equalized the variances of the strains tested. Prior to the transformation, the data were heteroscedostic, with the more resistant strains having the larger variances. A Bartlett's test for the homogeneity of the variances of the transformed data showed that they were homoscedastic ($\chi^2_{14} = 14.50$, $0.5 > p > 0.25$), despite the fact that the means ranged from 2.1 to 3.1 log time units (or from about 8 to 24 hours). (A $\sqrt{\mathrm{AST}}$ transformation gave a similar pattern of results.)

The data also seemed to be approximately normally distributed. A simple graphical test was run on the results from several strains, and no consistent departures from normality were noted.

The effect of mortality not due to DDT could be estimated because two series of flies were tested on clean petri plates. We concluded that this "natural mortality" (deaths not caused by DDT) did not have a significant effect on AST because the two causes of death were largely non-overlapping, that is, few, if any, flies had died due to natural mortality by the time most of the flies were dead due to DDT.

Nonchromosomal inheritance was investigated by a comparison of the means of the 8R and 8C strains. A t-test gave a result of $t(18\text{ df}) = 0.353$ $(0.8 > p > 0.5)$. This result showed no evidence of nonchromosomal effects.

The combined 8R and 8C results were compared to the 8T results. Again there was no significant difference with $t(37\text{ df}) = 0.251$ $(p > 0.8)$. This result indicated that the crossover suppressors were effective.

The genotypes in this type of chromosomal analysis are arranged to be analyzed in two substitution series that show the dominant and recessive effects and interactions of the three major chromosomes. Series I shows the recessive effects in the factorial substitution series HHH → RRR. Similarly, Series II shows the dominant chromosomal effects in a HHH → CCC substitution series. Each of these sets of eight genotypes is analyzed separately. The three major chromosomes are considered to be factors occurring at two levels, R and H in Series I, and H and C in Series II. This arrangement lends itself to a factorial analysis of variance.

A factorial analysis of variance in which the number of replicates is unequal is extremely laborious (SOKAL and ROHLF 1969). A complete set of 18 replicates for each genotype was chosen to be analyzed for Series I, and a complete set of 19 replicates was chosen for Series II. Extra replicates were eliminated by use of a random number table. Independent sets of 18 and 19 replicates were chosen from the 39 replicates of the HHH genotype (Strains 8R, 8C and 8T).

TABLE 1

Log transformed and untransformed mean Average Survival Times (AST) for the 15 genotypes tested for DDT resistance in the chromosomal analysis

Strain	Genotype	Transformed mean (ln hrs)	Untransformed mean (hrs)	Transformed variance
		Series I ($n = 18$ replicates)		
1	RRR	3.11972	23.7	0.0224
2	RHH	2.71452	15.8	0.0307
3	HRH	2.86425	18.7	0.0448
4	HHR	2.98952	21.3	0.0339
5	RRH	2.90942	19.3	0.0201
6	RHR	2.89182	19.1	0.0401
7	HRR	3.13794	24.2	0.0218
8*	HHH	2.57746	13.9	0.0404
		Series II ($n = 19$ replicates)		
8*	HHH	2.60794	14.3	0.0397
9	HCC	2.13979	8.8	0.0271
10	CHC	2.30222	10.9	0.0357
11	CCH	2.20067	9.3	0.0297
12	HHC	2.42070	11.6	0.0273
13	HCH	2.28853	10.1	0.0223
14	CHH	2.64434	14.8	0.0206
15	CCC	2.09756	8.3	0.0122

* Strain 8 of Series I and Series II differ slightly because they represent two independent samples drawn from the pooled 8R plus 8C plus 8T data.

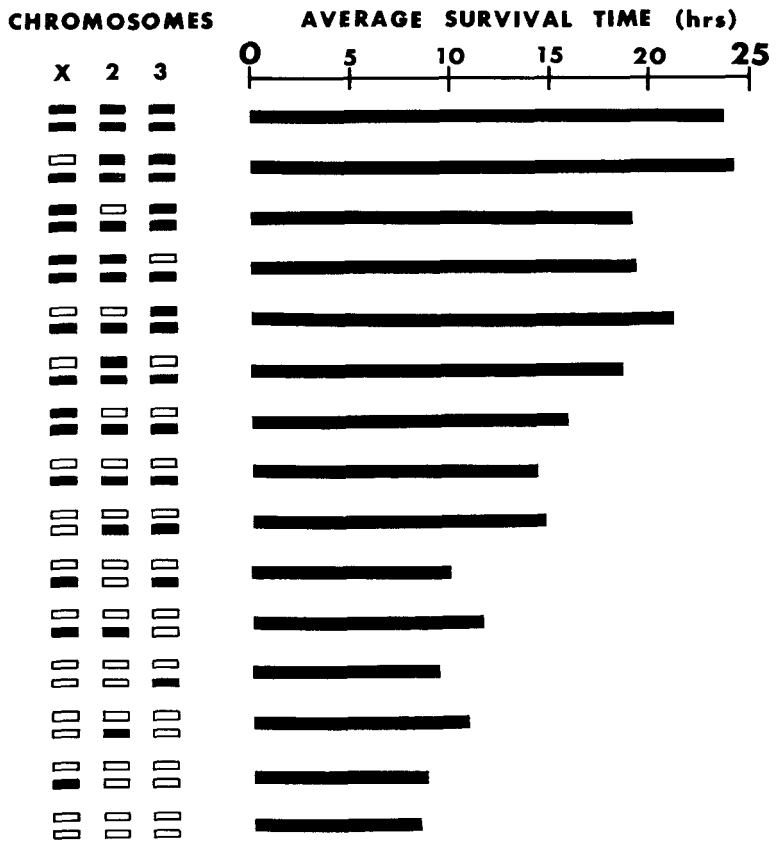

FIGURE 2.—The resistance of the genotypes tested in the chromosomal analysis. Solid chromosomes are from the DDT-resistant strain 91R and blank chromosomes are from the unselected control strain 91C.

The means and variances of the transformed data are given in Table 1. The means of the untransformed results are also included to give some idea of the actual differences in survival times of the various genotypes. Figure 2 depicts the untransformed results graphically.

Separate analyses of variance were performed on the Series I and Series II data. The results are presented in Tables 2 and 3. The four right hand columns of Table 2 are an analysis of variance table for the Series I data. This table shows that chromosomes *II* and *III* have very important effects on resistance and that there is a significant interaction between the first and third chromosomes. Table 3 similarly shows that all three chromosomes have significant effects on resistance, but that the second and third chromosomes are much more important sources of variation than is the first chromosome. There is also a significant interaction between the dominant effects of the second and third chromosomes.

The data of a factorial analysis of variance must be carefully examined when an interaction between two factors is present. The presence of a significant inter-

TABLE 2

Results of the factorial analysis of variance for the Series I data

Source of variation	Effect or interaction	Percent of total variance attributable to each effect or interaction	df	SS	MS	*F*
I	0.016579	0.22	1	0.00990	0.00990	1
II	0.214501	36.15	1	1.65640	1.65640	52.11**
III	0.268340	56.58	1	2.59224	2.59224	81.55**
I × *II*	—0.003103	0.01	1	0.00034	0.00034	1
I × *III*	—0.074537	4.37	1	0.20000	0.20000	6.29*
II × *III*	—0.026341	0.54	1	0.02497	0.02497	1
I × *II* × *III*	0.042840	1.44	1	0.06608	0.06608	2.08
Error			136	4.32331	0.03179	
Total	0.438279		143			

* Significant at the 0.05 level F (0.05, 1, 120) = 3.92.
** Significant at the 0.01 level F (0.01, 1, 120) = 6.85.

action in these experiments means that the effect of a chromosome on resistance is not constant. In other words, the effect of one chromosome depends on which other chromosomes are present in the genotype.

Interactions can be examined by means of a two-way table. Table 4 is a two-way table of the Series I data, which shows how the significant *I* × *III* interaction arises. In this table, the average effect of each chromosome at each level of the other chromosome can be examined. If no interaction were present, the effect of a chromosome, say the first, should be the same when the third chromosome is present at either the R or H level. The effect of a chromosome at a level can be calculated as the difference between the means at that level. For example at the

TABLE 3

Results of the factorial analysis of variance for the Series II data

Source of variation	Effect or interaction	Percent of total variance attributable to each effect or interaction	df	SS	MS	*F*
I	0.053041	1.92	1	0.10691	0.10691	3.99*
II	0.312166	66.46	1	3.70301	3.70301	138.02**
III	0.195301	26.01	1	1.44941	1.44941	54.02**
I × *II*	—0.012005	0.10	1	0.00547	0.00547	1
I × *III*	—0.027317	0.51	1	0.02835	0.02835	1.06
II × *III*	0.069378	3.28	1	0.18290	0.18290	6.82*
I × *II* × *III*	—0.050125	1.71	1	0.09549	0.09549	3.56
Error			141	0.02683	0.02683	
Total	0.540439		151			

* Significant at the 0.05 level F (0.05, 1, 120) = 3.92.
** Significant at the 0.01 level F (0.01, 1, 120) = 6.85.

TABLE 4

Two-way factorial ANOVA table for chromosomes I and III of Series I

		Factorial level of the first chromosome R--	H--	
Factorial level of the third chromosome	--R	RRR = 3.120	HRR — 3.138	
		RHR = 2.892	HHR = 2.990	
		R–R — 3.006	H–R = 3.064	--R — 3.035
		RRH = 2.909	HRH = 2.864	
	--H	RHH = 2.715	HHH = 2.577	
		R–H = 2.812	H–H = 2.722	--H = 2.767
		R-- = 2.909	H-- = 2.892	--- = 2.901

In this table the mean Average Survival Times (AST) of the 18 replicates for each genotype is given. R and H as in Figure 1. Various marginal means ASTs are also given. In the marginal genotypes (—) denotes an unspecified chromosome. For example, R–R is the average of the means of RRR and RHR. (Log time units).

R level of third chromosome, the effect of the first chromosome is (R-R minus H-R) = (3.006 − 3.064) = −0.058. At the H level of the third chromosome the first chromosome has a positive effect on resistance of (2.812 − 2.722) = +0.090. The fact that the R-- and H-- marginal totals are similar (and hence, there is no significant effect of the first chromosome) is seen to be due to small opposite effects on resistance rather than to a total lack of effect. Note that −(0.058 + 0.090)/2 = 0.074 the value that appears as the $I \times III$ interaction in Table 2.

A similar examination of the effect of the third chromosome shows that it has a net positive effect at each level of the first chromosome, but that the effect at the H level (0.342) is much greater than the effect at the R level (0.194). Thus, each resistant chromosome has a negative influence on the other resulting in a relative decrease in the effect of the other chromosome.

In spite of the interaction, it seems safe to say that the first chromosome has a relatively minor effect on resistance compared to the effect of the third chromosome. The third chromosome has large effects on resistance but these vary in magnitude with the level of the first chromosome.

The source of the $II \times III$ interaction can be analyzed by the same method used in Table 4. Such an analysis shows that at the C level of the third chromosome, the second chromosome has an effect of 0.243, while at the H level it has an effect of 0.381. Similarly, the third chromosome has an effect of 0.126 at the C level of the second chromosome and an effect of 0.264 at the H level. This situation differs from the interaction between the first and third chromosomes of Series I. In this case, there is a positive interaction with the presence of one chromosome enhancing the expression of the other. The interaction arises out of a variation in the magnitude of the effect of a chromosome, which depends on the presence or absence of another resistant chromosome in the genotype. The possible effect of the scale of measurement on these interactions, as well as their possible genetic significance, will be explored in the discussion.

The magnitude of the effect or interaction of each source of variation is presented in the second column of Tables 2 and 3. The percent of the total variance attributable to each effect or interaction is given in the third column of each table.

In each series the effects of chromosomes *II* and *III* are more important than those of the first chromosome. In addition, there seems to be a large difference in the relative importance of the effects of the second and third chromosomes, with chromosome *III* being more important in Series I and less important in Series II. All of the single chromosome interactions in both series are negative except for the *II* × *III* interaction in Series II. The possible significance of this observation will be considered in the discussion.

DISCUSSION

Much of the interest in the genetics of DDT-resistance in the 91R population comes from its long history of selection with DDT and the high level of resistance it has achieved. This population had been exposed to increasing amounts of DDT for 23 years at the time of the experiment. C. LAURIE (personal communication) has conservatively estimated that this represents at least 350 generations of selection for DDT resistance.

The level of DDT resistance reported for most other strains of *Drosophila melanogaster* often seems to have been slight, usually a few times that of the controls. CROW (1957a) even suggested that nonspecific "vigor tolerance" might be involved. The high level of larval resistance reported by the Japanese workers in their strain seems to be an exceptional case (OGAKI and TSUKAMOTO 1953; KIKKAWA 1958).

It is difficult to compare DDT-resistance measured by different methods and at different times, but an attempt will be made to demonstrate the high level of resistance of the 91R population. Two previous studies of the relative resistance of 91R and 91C were conducted by MERRELL (1960) and UNDERHILL and MERRELL (1966). In the 1960 study, 91R had an ED_{50} of 11.7 mg, while 91C had an ED_{50} of 0.16 mg. The 1966 tests gave values of 2.6 mg and 0.036 mg, respectively. Thus, in two separate tests 91R was 72.5 and 73.1 times more resistant than 91C. In 1974 these tests were repeated with 100 mg plates at the same temperature (21°). Few flies were killed in these tests, indicating an ED_{50} much greater than 100 mg and a greatly increased level of resistance. This lack of mortality made it necessary to change the method of testing to the time-based test used in this study.

It is important to keep two things in mind in the discussion of the results of the chromosomal analysis. First, a whole chromosome from 91R or 91C is the basic unit of analysis. Any effect, dominance interaction, or interchromosomal interaction observed is an average of the effects of all of the resistance genes on the chromosomes. Second, if there was a significant amount of variability for resistance in either population, an average has been taken by the sampling procedure, which extracted and tested a large number of different chromosomes from each population.

The results of this chromosomal analysis are similar to those reported for several other DDT-resistant Drosophila strains. In general, DDT-resistance was found to be multifactorial and to involve genes of intermediate dominance (producing partially resistant heterozygotes). In this study both the second and third chromosomes had major effects on resistance, while the first chromosome showed a much smaller but statistically significant dominant effect. The second and third chromosomes both had substantial dominant and recessive effects, although these were unequal. These results indicate multiple resistance genes of intermediate dominance.

The same sort of inheritance of resistance was reported by BOCHNIG (1954) on three strains and by KING (1954, 1955, 1956) on two strains. These authors used traditional F_1, F_2, backcross analyses. KING and SOMME (1958), CROW (1957a,b) and OSHIMA (1958) conducted chromosomal analyses of resistant strains with generally similar results. CROW (1957a,b) carried his analysis further and attempted to determine the number of factors responsible for resistance on the second and third chromosomes. His analysis was for dominant resistance effects only. The analysis of the third chromosome was inconclusive, but the analysis of the second chromosome seemed to show resistance factors concentrated in two areas adjacent to the centromere. The rest of the second chromosome had relatively unimportant effects on resistance.

Several Japanese workers, other than OSHIMA (1958), have reported basically different results. OGAKI and TSUKAMOTO (1953), TSUKAMOTO and OGAKI (1953) and KIKKAWA (1958) found that most DDT-resistance in their two strains of *Drosophila melanogaster* was due to a single chromosome region on the second chromosome (near scabrous, *II*-66.7). However, other resistance genes with minor effects were probably present on the first and third chromosomes (KIKKAWA 1958). The number of resistance factors was probably underestimated, since their test for resistance involved a single high dose that may have allowed only those flies with the major resistance region to survive. At lower doses, more genes might have been detected. This resistance region also contrasts with the other instances of resistance in that it is apparently completely dominant. A possible explanation for these results is that this single, dominant region confers larval resistance; all other studies have focused on genes conferring resistance on adults.

The final area to be discussed is the interchromosomal interactions that were detected in this chromosomal analysis. The basic idea of nonallelic interaction is that the magnitude of the effect of an allele at one locus is dependent on the alleles present at other loci (MATHER and JINKS 1971). This concept is a statistical one and involves the comparison of the observed effect of two or more genetic units with an expected effect based on the assumption of additive effects. The difference observed between these two quantities is the interaction.

The reason for considering nonallelic interactions is that they can have important effects on the measurement and on the evolution of resistance. In terms of measuring resistance, interactions are important because the effect of a genetic unit is no longer constant if they are present. The genetic background must be

specified before the effect of a genetic unit can be stated. From an evolutionary point of view, interactions are one way in which the value of a selected characteristic can be increased by selection. New genetic combinations that interact positively can be picked out and concentrated by selection. Also, long-term selection might deplete additive genetic variation in a population, thus making interacting genes an important source of selective advance (CROW and KIMURA 1965).

The detection of interaction depends on measuring the effect of one genetic unit in the presence or absence of another genetic unit, which can easily be done in a factorial chromosomal analysis. However, the results are complicated because both the magnitude and the occurrence of interaction can be changed by transforming the scale on which measurements are made (MATHER and JINKS 1971). The appropriateness of a scale of measurement must be determined on some other basis. Often a search is made for a scale that causes the interactions to disappear. If such a scale is found, and it does not complicate the analysis in other ways, it can be concluded that interactions need not be included in an analysis of the data. It is difficult, however, to show that genetic interactions are present in a set of data and are not actually a statistical, scaling artifact.

CROW (1957a,b) and KING and SOMME (1958) performed factorial chromosomal analyses that could be examined for the presence of interactions. They found that on the scale of measurement used, interactions between chromosomes were unnecessary to explain the data. The scales of measurement were found to be otherwise adequate.

In our chromosomal analysis two significant first-order interactions were found. In the Series I data, a significant, negative $I \times III$ interaction was found. In Series II, a significant positive $II \times III$ interaction was detected. Both have a p value near 0.01, so that it seems unlikely they are due to chance.

It is interesting that five of the six first-order interactions were negative, although the $I \times II$ interaction in Series I was near zero. One possible explanation for these data might be that the negative interactions resulted from the test conditions. A negative interaction arises when the combined effects of two genetic units are less than the sum of their individual effects. This decrease might be due to the increased homozygosity of the more resistant "combined" genotypes. This explanation is unsatisfactory, however, because negative interactions were found both in Series I and Series II results, while the combined genotypes were more homozygous in Series I only. A second explanation for the presence of the negative interactions might be a relative change in the resistance of the more resistant flies due to the test conditions. Less resistant flies might survive as long as their level of resistance permits, but the progressive starvation and dehydration of more resistant flies may cause them to die sooner than expected, due to decreased inherent resistance.

Possibly, a transformation could be found that would increase the relative resistance of the more resistant genotypes in each series more than it affected the less resistant genotypes. Such a transformation would be expected to increase the relative importance of the $II \times III$ positive interaction, even though it tended

to remove the negative interactions. Hence, if our interpretation of the negative interactions is correct, the positive interaction might represent a real genetic interaction with some physiological basis. Experiments are now underway to explore this possibility. As support for this idea, it should be noted that the positive interaction occurs between the two chromosomes with large effects on resistance. Perhaps a similar positive interaction in the Series I data was masked by the negative interaction due to the highest levels of resistance.

This chromosomal analysis, then, gave a picture of resistance similar to that reported by most other workers with DDT resistance in Drosophila. Thus, these results indicate that the higher levels of resistance produced by long-term selection were not brought about by radically different genetic mechanisms such as the single genes of major effect that are often found to be responsible for resistance in other insect species (see review in BROWN, 1967). Since resistance has apparently increased greatly since 1966, it seems that there was no insurmountable selection plateau in the 91R population at that time.

Nevertheless, in these data there are indications that the number of important resistance genes is not large. For example, the first chromosome in the 91R did not contribute an important amount to resistance compared to the second and third chromosomes. The second and third chromosomes showed large differences in resistance between the Series I and Series II data, with the third chromosome being more important than the second in Series I and the reverse being true in Series II. Apparently, the resistance factors on the second chromosome had considerably greater average dominance than do those on the third chromosome. These large differences between chromosomes were not what one would expect if the average effects of many resistance factors, randomly spread over the chromosomes, were being observed.

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