HETEROSIS IN DDT RESISTANT AND SUSCEPTIBLE POPULATIONS OF DROSOPHILA MELANOGASTER

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THE phenomenon of heterosis has generally been studied in relation to some arbitrarily selected trait such as size, weight, or yield. Heterosis is then regarded as being manifested by an increase in the chosen trait. Although various definitions have been proposed (GOWEN 1952), heterosis is most commonly and most meaningfully defined as an increase in the chosen trait in crossbred individuals as compared to the better of their more inbred parents. While heterosis is usually considered to affect all of the characters of hybrids, it is actually only measured in relation to the trait or traits selected for study. Recently DOBZHANSKY (in GOWEN 1952) has equated heterosis with adaptive value and has called increased size or vigor "luxuriance" when it does not contribute to fitness. This usage, while it has the advantage of referring always to the same trait, adaptive value, may lead to considerable confusion since it is entirely possible for smaller size, for example, to confer greater adaptive value than large, and hence a direct contradiction between the historical meaning of the word (SHULL 1948) and this new definition will result. The experiments to be reported were designed to test some of the concepts which have grown up around the phenomenon of heterosis.

METHODS

The flies used in the experiments were drawn from the two most resistant stocks (731 R and 91 R) among several developed during six years of exposure to DDT. These populations had been maintained in "population bottles" (REED and REED 1948) in which were placed slips of filter paper impregnated with DDT. In this way all stages of the life cycle were likely to be exposed. Separate unexposed controls (731 C and 91 C) were run for each population. At the time of the crosses each of these four stocks had formed an isolated breeding population for a period of six years. These strains were both descended from flies captured in wild populations of *Drosophila melanogaster* in St. Paul, Minnesota, in the summer of 1952. Large numbers of flies rather than single inseminated females were collected in an effort to have initial populations with considerable genetic variability. (MERRELL and UNDERHILL 1956, gave the size of the original populations as up to 50 flies, but a check of the notes made at the time of collection indicates that for these two populations it was more like a few hundred.) Their heterozygosity was indicated by the continued increase in resistance of the exposed populations. This increase was evidenced by the need to increase the dose to the populations from 0.5 mg to 40 mg during this period as well as by the separate tests run to deter-

mine the ED_{50}. For further details on the methods used and the results with inbred and laboratory stocks see MERRELL and UNDERHILL 1956.

The following types of flies were tested:

	Control	Resistant
P_1	731 C	731 R
	91 C	91 R
F_1	731 C ♀♀ × 91 C ♂♂	731 R ♀♀ × 91 R ♂♂
	91 C ♀♀ × 731 C ♂♂	91 R ♀♀ × 731 R ♂♂
F_2	731 C ♀♀ × 91 C ♂♂	731 R ♀♀ × 91 R ♂♂
	91 C ♀♀ × 731 C ♂♂	91 R ♀♀ × 731 R ♂♂

The traits which were measured were DDT resistance, fecundity, and fertility. All of the tests for a given generation were run concurrently, and all of the generations were run in a little over a month in a constantly lighted room held at 21 ± 1°C and 50 percent relative humidity so that comparisons within and between generations should be reasonably valid.

The flies for testing and for making the crosses were derived from four half-pint culture bottles for each original population. Each of the four bottles contained as parents 20 females and 20 males taken from the population bottles. No effort was made to get virgin females so that the flies in each bottle were descended from at least 40 parents and possibly quite a few more. Hence, the flies from each population used in the experiments came from at least $4 \times 40 = 160$ parents and probably more. In this way it was hoped that a broad sample of the gene pool of each population would be obtained. The progeny from these four cultures were collected twice daily and were mixed before flies were taken for use in the crosses or in the various tests.

Reciprocal crosses were made between 731 C and 91 C and between 731 R and 91 R, four bottles for each cross, with 20 virgin females and 20 males per bottle, again a total of 160 parents for each F_1 population. The four F_1 bottles for a particular cross (e.g., 91 C × 731 C) served as the source of flies for the tests and of the 160 flies which served as parents of the 91 C × 731 C F_2 generation.

For the tests of DDT resistance, only females were used (See MERRELL and UNDERHILL 1956 for further details). The test period had to be extended to 24 hours from the six hours previously used in order to kill enough flies of the resistant stocks to get an estimate of the ED_{50}.

For the fecundity tests eight to ten females and twice as many males were placed in a fresh bottle of food for three days. On the fourth day five of these females were placed individually in creamers containing two males apiece and food darkened with India ink. The flies were transferred every 24 hours to a fresh creamer to get the egg production on the fourth, fifth, and sixth day after eclosion. These 72-hour tests were run for 30 females of each stock, with five females of each of the four stocks being started each day for six consecutive days. In order to test fertility these cultures were set aside for 15 days at which time the adults were counted. A second count was made after two more days as a check for late

emerging flies, and the creamers were then discarded. The possibility of a negative correlation between fecundity and fertility was checked because of the possibility that the food in creamers containing large numbers of larvae might be exhausted and thus a false picture of fertility be presented. However, a plot of fertility against fecundity indicated no such correlation. Fertility, of course, was influenced by the genotypes of the offspring as well as those of both parents and was, therefore, a more complex trait than either fecundity or DDT resistance which, as measured here, were essentially traits only of the females.

RESULTS

Tables 1 and 2 show the results of the tests for DDT resistance in the resistant and control populations and in their F_1 and F_2 hybrids. The ED_{50}'s in Table 2 were estimated by the SPEARMAN-KÄRBER method (See FINNEY 1952; KEMPTHORNE, BANCROFT, GOWEN, and LUSH 1954). As a check, probit analyses by the arithmetical method (FINNEY 1952) were also calculated for the resistant stocks. Three of the six probit regression lines showed a significant χ^2 tests for hetero-

TABLE 1

DDT resistance of control and resistant populations and F_1 and F_2 hybrids (24 hr test)

	Control					
	P_1		F_1		F_2	
	731 C	91 C	731 C × 91 C	91 C × 731 C	731 C × 91 C	91 C × 731 C
N*	125	125	125	125	123	113
Conc DDT (mg)	Percent down†	Percent down	Percent down	Percent down	Percent down	Percent down
100.0	100.0	100.0	100.0	100.0	100.0	100.0
10.0	100.0	100.0	100.0	100.0	99.2	100.0
1.0	100.0	97.6	100.0	100.0	98.4	99.1
0.1	89.6	32.8	30.4	31.2	52.0	64.6
0.01	6.4	0.0	8.0	4.8	7.3	6.2
	Resistant					
	P_1		F_1		F_2	
	731 R	91 R	731 R × 91 R	91 R × 731 R	731 R × 91 R	91 R × 731 R
N*	125	125	125	125	127	137
Conc DDT (mg)	Percent down	Percent down	Percent down	Percent down	Percent down	Percent down
100.0	84.8	68.8	68.0	68.8	78.0	85.4
10.0	67.2	52.8	42.4	48.0	57.5	53.3
1.0	27.2	17.6	5.6	4.8	22.8	28.5
0.1	7.2	1.6	4.8	4.8	9.4	8.0
0.01	1.6	2.5‡	1.6	2.4	1.6	3.6

* Number at each concentration.
† Includes all flies unable to stand, both knockdown and kills.
‡ N=120.

TABLE 2

ED_{50} for resistant and control populations and F_1 and F_2 hybrids

	Population	Log ED_{50} (Hundredths of mg)		ED_{50} (mg)	Relative resistance
		SPEARMAN-KÄRBER	Arith. probit analysis		
P_1	731 C	1.54 ± .04	.....	0.04	1.
	91 C	2.20 ± .04	.....	0.16	4.
	731 R	3.62 ± .07	3.67 ± .08	4.2	105.
	91 R	4.07 ± .07	4.18 ± .09	11.7	293.
F_1	731 C × 91 C	2.12 ± .04	.....	0.13	3.2
	91 C × 731 C	2.14 ± .05	.....	0.14	3.5
	731 R × 91 R	4.28 ± .07	4.41 ± .10	18.9	473.
	91 R × 731 R	4.21 ± .07	4.34 ± .10	16.3	407.
F_2	731 C × 91 C	1.93 ± .06	.....	0.08	2.0
	91 C × 731 C	1.80 ± .05	.....	0.06	1.5
	731 R × 91 R	3.81 ± .07	3.88 ± .09	6.4	160.
	91 R × 731 R	3.71 ± .07	3.76 ± .08	5.2	130.

geneity, but in each case they were due to a large contribution from classes with small expectations. The log ED_{50}'s obtained by the two methods were in good agreement. Most of the control data, however, was not well suited for probit analysis. While the SPEARMAN-KÄRBER estimates may lack some precision, the essential features of the data are such that these estimates are quite adequate.

There was no heterosis for resistance in the F_1 controls while in the F_2 the ED_{50} was lower than in the F_1, but not lower than that of the lower parent. The survival of a few F_2 flies at the higher concentrations may indicate that segregation and recombination had produced a few individuals with increased resistance.

The resistant F_1 showed marked heterosis for resistance. The F_2 showed a significant decrease in resistance as compared to the F_1, but fell between the original parental populations in resistance. There is no significant difference between the results from reciprocal crosses in either the control or resistant crosses, except possibly in the control F_2 where $P = 0.05$.

The results of the tests for fecundity are shown in Table 3. It is noteworthy that every female tested laid at least some eggs so that the large variance is due to the variation among females in the number of eggs they produced rather than to some females who laid no eggs at all. Also noteworthy is the low fecundity of the 91 R population, significantly lower than that of 91 C, 731 C, and 731 R, which did not differ significantly from each other.

Significant heterosis for fecundity was observed in the F_1 in both resistant and control hybrids. Surprisingly, in the F_2 only one out of the four populations declined significantly below the fecundity of the F_1 from which it was derived.

The results on fertility (Table 4) are not particularly striking. However, again it is noteworthy that only one female, which laid 133 eggs, failed to produce adult

offspring. The most unusual finding was the low fertility (70.8 percent) of the 731 C females. This reduced fertility was not due to low fertility in just a few females, for only one 731 C female had fertility lower than 50 percent. Furthermore, crowding in the creamers could hardly have been a factor, for the 91 C females, which were tested concurrently in the same batch of creamers, did not differ significantly from the high fecundity of the 731 C females, yet had a high fertility (90.6 percent).

Table 5 summarizes the data for purposes of comparison. The original control populations differed significantly in both resistance and fertility. The control F_1 showed heterosis only for fecundity, not for resistance or fertility. The resistant populations differed significantly from each other in resistance and in fecundity and were approximately 75 and 100 times more resistant than their respective

TABLE 3

Fecundity

Controls			Resistant		
	N females	Eggs/female/72 hr		N females	Eggs/female/72 hr
P			P		
731 C	30	145.3 ± 9.6	731 R	29	138.8 ± 7.5
91 C	30	143.7 ± 7.3	91 R	29	107.2 ± 8.4
F_1			F_1		
731 C × 91 C	30	172.7 ± 8.8	731 R × 91 R	30	163.8 ± 7.4
91 C × 731 C	30	180.4 ± 6.4	91 R × 731 R	30	150.5 ± 6.6
F_2			F_2		
731 C × 91 C	30	174.8 ± 8.6	731 R × 91 R	30	146.4 ± 8.6
91 C × 731 C	30	180.6 ± 9.0	91 R × 731 R	30	145.8 ± 6.5

TABLE 4

Fertility as adults/eggs laid

Controls			Resistant		
	Total eggs	Percent fertility		Total eggs	Percent fertility
P			P		
731 C	4,360	70.8	731 R	4,027	82.0
91 C	3,907	90.6	91 R	3,109	82.8
F_1			F_1		
731 C × 91 C	5,182	87.4	731 R × 91 R	4,916	92.9
91 C × 731 C	5,412	87.9	91 R × 731 R	4,526	90.3
F_2			F_2		
731 C × 91 C	5,245	83.1	731 R × 91 R	4,391	90.9
91 C × 731 C	5,417	91.7	91 R × 731 R	4,374	86.6
Total	29,523			25,343	

TABLE 5

Comparison with respect to DDT resistance, fecundity, and fertility

Control				Resistant			
	Resistance ED_{50} (mg)	Fecundity (eggs/ female/ 72 hr)	Fertility percent		Resistance ED_{50} (mg)	Fecundity (eggs/ female/ 72 hr)	Fertility percent
P				P			
731 C	.04	145.3	70.8	731 R	4.2	138.8	82.0
91 C	.16	143.7	90.6	91 R	11.7	107.2	82.8
F_1				F_1			
731 C × 91 C	.13	172.7	87.4	731 R × 91 R	18.9	163.8	92.9
91 C × 731 C	.14	180.4	87.9	91 R × 731 R	16.3	150.5	90.3
F_2				F_2			
731 C × 91 C	.08	174.8	83.1	731 R × 91 R	6.4	146.4	90.9
91 C × 731 C	.06	180.6	91.7	91 R × 731 R	5.2	145.8	86.6

controls. The resistant F_1 was heterotic for all three traits. While some decreases occurred in the F_2's, in no case was any value found which was lower than that of the lower original parent. While both resistant and control crosses showed heterosis for fecundity, the fecundity of the controls was generally higher than that of the resistant populations.

DISCUSSION

Although only two gene pools were present in duplicate in 1952, after six years of isolation and selection, four distinct populations had evolved. Each differed significantly from each of the other three populations in at least two of the three traits tested. No obvious relationship among these traits is apparent, for the most resistant population (91 R) had the lowest fecundity, the most fecund (731 C) had the lowest fertility, and the most fertile (91 C) was not resistant to DDT.

The most striking heterosis is found in the fecundity of the F_1 controls and in the DDT resistance of the resistant F_1. These results suggest a relationship between previous selection pressures and the degree of heterosis observed. The constant heavy selection pressure exerted by DDT in the resistant populations was absent in the control populations, and only in the F_1 resistant hybrids was heterosis for resistance observed. Similarly, while fecundity must have been important in both types of populations, it can be assumed to have been of relatively greater importance in the absence of DDT so that the higher fecundity and greater degree of heterosis for fecundity in the control populations is perhaps not surprising.

Theories to account for heterosis have been of two major types, which may, for convenience, be termed dominance (JONES 1917) and overdominance (EAST 1936; HULL 1945). Heterosis due to overdominance is postulated to stem from heterozygosity *per se*. The greater the number of heterozygous loci, therefore, the greater the heterosis expected. Under a strict interpretation of this theory, only the interactions within loci are significant as causes of heterosis.

Under the dominance theory, the genes favorable to increased size or vigor are assumed to be dominant or at least partially dominant. The hybrid will have a greater number of loci with at least one favorable dominant than either parent. Therefore, the minimum number of loci required for the expression of this type of heterosis is two. This type of heterosis may be due simply to the masking of deleterious recessive genes, but it may also be due to nonallelic interactions among the favorable genes. Theoretically, homozygotes equal to or even superior to the heterozygotes should be obtainable, but with many loci involved, the problem of obtaining homozygosity for only the favorable genes becomes extremely complex.

While these theories are often regarded as alternatives, they are by no means mutually exclusive, and the heterosis observed in any particular case may well be due to the masking of deleterious recessives and to favorable interactions, both nonallelic and allelic. It is, moreover, entirely conceivable that in different instances of heterosis, the relative importance of these different factors may vary widely. Furthermore, while the theories are clear-cut, the experimental determination of which mechanism is more important in a given situation is not a simple matter, for ideally it requires a knowledge of the behavior of genes at individual loci in a polygenic system.

In these experiments the data indicate that a finding of heterosis in one trait does not necessarily mean that all traits of the same organism will also manifest heterosis. Here, then, heterosis appears not to be some sort of generalized phenomenon resulting simply from heterozygosity, but must be determined by the genetic situation at the loci which determine the trait or traits under study.

Recent work with natural populations has led to the development of the theory of coadaptation or the integration of the genotype. Natural selection is thought to operate within a breeding population to build up "harmoniously integrated genetic systems." Three levels of integration have been postulated (WALLACE and VETUKHIV 1955): "(1) Integration based upon epistatic interactions between homozygous loci. (2) Coadaptation of different gene arrangements within local populations involving both heterosis and epistasis, and (3) the integration of entire gene pools through selection for heterozygosity." These statements indicate the significance of heterosis to the theory of coadaptation and would seem to imply that overdominance is the mechanism considered to be responsible for the heterosis at the third level (WALLACE 1958).

The considerable and continued increase in resistance in the exposed populations is evidence for the genetic variability of the original populations since it has already been demonstrated (MERRELL and UNDERHILL 1956) that selection for DDT resistance is ineffective in highly inbred stocks and even, in six of seven tested, in several well-known "wild" stocks of *D. melanogaster* which have been maintained as laboratory stocks for many years, and are also presumably quite inbred and relatively homozygous. Therefore, since each population has been maintained as a separate breeding unit consisting of a few hundred individuals confined for more than six years in a very small space, it would seem that conditions were very favorable for the development of coadaptation. However, the

results of the crosses indicate that such integration of the genotypes has not taken place within these populations. The maximum DDT resistance was found in F_1 resistant hybrids and the maximum fecundity in the F_1 control hybrids. In other words, the maxima were found where there should have been minimum coadaptation. This result is frequently reported in the work on coadaptation and would seem to be its most serious weakness. For all three levels of integration discussed by WALLACE and VETUKHIV in 1955, the F_1 hybrids from crosses between individuals from two different breeding populations representing two different integrated gene pools would be expected to be less viable than the parents. The fact that they generally are heterotic requires that some explanation other than coadaptation be invoked to explain this heterosis. Therefore, the heterosis of F_1 interpopulation hybrids represents a serious flaw in the whole argument for coadaptation. Furthermore, since none of the F_2's fell below its lower parent in DDT resistance, fecundity, or fertility, there was no evidence of "hybrid breakdown" in the F_2 in these experiments.

Since coadaptation does not seem to give a satisfactory interpretation for the data, other possibilities may be suggested. The simplest perhaps is that strong selection for DDT resistance in the exposed heterozygous populations led to an increase in the frequency of genes favoring resistance. Somewhat different arrays of genes for resistance accumulated in 91 R and 731 R so that the F_1 hybrids carried a number of such genes whose combined effects conferred maximum resistance. In the unexposed controls no such accumulation had occurred, and the F_1 hybrids had intermediate resistance. Similarly, since fecundity was of relatively greater importance in the control populations, genes favoring increased fecundity were more strongly favored by selection, and in the F_1 hybrids their combined action led to the high fecundity observed. Though favored to some extent by selection in the exposed populations, fecundity was not of the same relative importance as in the controls so that the parental populations and their hybrids were less fecund than the comparable control populations due to the smaller accumulation of genes favorable to fecundity in the resistant populations. Similar reasoning may be applied to the fertility of the resistant flies, but while 91 C had a high fertility, the low fertility of 731 C, which appears to be real and not an artifact in the data, presents a puzzle for which there appears to be no simple answer.

The suggested explanation, of course, follows the lines of the dominance hypothesis described earlier, with the genes for greater resistance or fecundity at least partially dominant, and the heterosis being due to additive effects or to interactions among those genes. The absence of heterosis for resistance in the control F_1 hybrids is then attributable to the fact that genes for resistance have not increased in frequency due to selection in these populations.

SUMMARY

Flies drawn from two DDT resistant populations (731 R and 91 R) were crossed, and the original populations and their F_1 and F_2 hybrids were tested for DDT resistance, fecundity, and fertility. Similar crosses and tests were made

with the controls (731 C and 91 C). Heterosis was observed in the resistant F_1 hybrids for all three traits but only for fecundity in the F_1 control hybrids. F_2 values were in some cases significantly lower than the F_1, but there was no indication of "hybrid breakdown" in the F_2. Since each of the four originally heterozygous populations had been maintained in isolation for six years, evidence was sought for the development of coadapted genetic systems during this period, but no indications of coadaptation were found.

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LITERATURE CITED

EAST, E. M., 1936 Heterosis. Genetics **21**: 375–397.

FINNEY, D. J., 1952 A statistical treatment of the Sigmoid response curve. *Probit Analysis*. 2nd Ed. Cambridge University Press. Cambridge, England.

GOWEN, J. W., 1952 *Heterosis*. Iowa State College Press. Ames, Iowa.

HULL, F. H., 1945 Recurrent selection for specific combining ability in corn. J. Am. Soc. Agron. **37**: 134–145.

JONES, D. F., 1917 Dominance of linked factors as a means of accounting for heterosis. Genetics **2**: 466–479.

KEMPTHORNE, O. T., T. A. BANCROFT, J. W. GOWEN, and J. L. LUSH, 1954 *Statistics and Mathematics in Biology*. Iowa State College Press. Ames, Iowa.

MERRELL, D. J., and J. C. UNDERHILL, 1956 Selection for DDT resistance in inbred, laboratory, and wild stocks of *Drosophila melanogaster*. J. Econ. Entomol. **49**: 300–306.

REED, S. C., and E. W. REED, 1948 Natural selection in laboratory populations of Drosophila. Evolution **2**: 176–186.

SHULL, G. H., 1948 What is "heterosis"? Genetics **33**: 439–446.

WALLACE, B., 1958 The average effect of radiation-induced mutations on viability in *Drosophila melanogaster*. Evolution **12**: 532–556.

WALLACE, B., and M. VETUKHIV, 1955 Adaptive organization of the gene pools of Drosophila populations. Cold Spring Harbor Symposia Quant. Biol. **20**: 303–310.