THE EFFECTS OF HETEROZYGOSITY CHANGES ON VIABILITY IN *DROSOPHILA MELANOGASTER*¹

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ABSTRACT

The viability effects of chromosomes from an old and from a new laboratory strain of *D. melanogaster* were studied in eight factorial combinations and at two heterozygosity levels. The combinations were so constructed that heterozygosity level could be varied in the third chromosomes of the carriers of a homozygous lethal marker, in the third chromosomes of their wild-type segregants, and in the genetic backgrounds of both. Excluding the effect of the marker and the exceptional outcomes of two of the combinations, and taking into account both large and small deviations from theoretical expectation, the following summary is given as the simplest consistent explanation of the results: 1) If total heterozygosities of two segregant types tend toward equality their viabilities tend toward equality also, whether background heterozygosity is high or low; if background heterozygosities is higher the tendency toward equality is slightly greater. 2) If total heterozygosity of two segregant types are unequal the less heterozygous type has the lower viability; the difference is more pronounced when background heterozygosity is low, less when it is high. 3) Differences between segregant viabilities are correlated with differences between the total heterozygosities of the two segregants; genetic background is effective to the extent, and only to the extent, that it contributes to the magnitude of this difference. This in turn appears to underlie, at least partly, the expression of a pronounced interchromosomal epistasis. Thus in this study viability is seen to depend upon both the quantity and distribution of heterozygosity, not only among the chromosomes of an individual but among the individuals of a given combination as well.

BALANCED polymorphisms in natural and experimental populations may depend for their expression upon a number of factors, among the most important of which is heterozygote superiority, or heterosis. The expression of such heterosis may be said to depend upon the quantity and distribution of heterozygosity among the chromosomes of an individual and among the individuals of a population. It is therefore pertinent to understand more precisely just how variations of quantity and distribution of heterozygosity relate, through heterosis, to the expression of this particular class of polymorphisms. The problem is complex and

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the results of its study varied, as the literature shows (CROW 1968; DOBZHANSKY 1964; DOBZHANSKY and SPASSKY 1968; MUKAI 1969a, 1969b; MUKAI and YAMAZAKI 1968; WALLACE 1963; and others). This variation may result from differences in the nature of the biological materials and/or differences in methodology (DOBZHANSKY and SPASSKY 1968), for the problem has been attacked from many points of view and at many genetic levels. Each investigation provides its own particular aspect of information.

The purpose of the present study is to examine the viability effects of high and low levels of heterozygosity in the third chromosomes of the carriers of a homozygous lethal marker, in the third chromosomes of the phenotypically wild-type competitors of these carriers, and in the genetic backgrounds of both. Such conditions provide eight theoretically possible heterozygosity combinations (Table 5) and, in the words of GREENBERG and CROW (1960), make possible a "... comparison of homozygous and heterozygous chromosomes with different levels of background heterozygosity in the other chromosomes. . . ."

MATERIALS AND METHODS

Four different lines, two basic and two derived, all maintained by mass culture, were used in this work:

Drop (35-Canton-S): The Drop eye mutant (LINDSLEY and GRELL 1968) used as a marker had its origin by X ray in a Canton-S line (KRIVSHENKO 1954). It is a homozygous lethal, is located at 3-99.2, and is not associated with any visible chromosomal rearrangements. Viability and fertility of the heterozygotes (*Dr/+*) are excellent. The trait shows remarkably little individual or age variation and is expressed to the same degree in both sexes. To place *Dr* in the Canton-S strain of this laboratory, *Dr/In(3L)P,In(3R)C* females were crossed to Canton-S males; *Dr* progeny were separated and maintained by mass culture and selection for over 227 generations. Following this, *Dr* females were backcrossed to Canton-S males repeatedly, generation after generation, for 35 additional generations, to produce the line designated above. In what follows, S designates a Canton-S chromosome and S° a Canton-S chromosome carrying *Dr*.

Wawawai: A wild-type strain collected at Wawawai, Washington on 27 September 1964, and maintained by mass culture, was used to provide a heterozygosity contrast. In what follows, W designates a Wawawai chromosome.

Chromosomal interchange lines, S/S;W/W and W/W;S/S: By means of a balanced lethal inversion stock, *SM1/Pm;TM6/Dl'* (LINDSLEY and GRELL 1968), two chromosomal interchange lines were constructed by the method shown in Figure 1. The S/S;W/W line has Canton-S second chromosomes and Wawawai third, and the W/W;S/S line the contrary. Canton-S flies used were the non-Drop segregants from the Drop (35-Canton-S) line. The small 4th chromosomes were disregarded and the X chromosomes, uncontrolled, were estimated to consist of material from the double balancer, Wawawai, and Canton-S lines in the ratio of 4:1:1, approximately. In the following, X designates an X chromosome of this type.

The crosses used to produce the eight heterozygosity combinations (Table 1) were made in such a way as to yield a theoretical 1:1 expectation of Drop to wild-type progeny and so that in the progeny all chromosomes would be X or S or W types, i.e., no interstrain crossover types would occur. The male parents for crosses 3, 4, 5 and 6 were obtained from the progeny of cross 7. These kinds of crosses are useful not only for their genetic simplicity but also for their provision of maximum heterozygosity differences. They also provide the desired levels of heterozygosity for the third chromosomes of the *Dr* carriers, for the third chromosomes of the wild-type flies, and for their genetic backgrounds, while at the same time they insure heterozygosity at but one level in the backgrounds of the two classes of flies in a given combination. No information is obtained from these experiments on the possible competitive effects of S°/S° larvae, but GRAHN

FIGURE 1.—Construction of chromosomal interchange lines; superscript omitted from $D1^7$.

(1969) has found that the mortality of these homozygotes, though alterable by genetic background, is greatest during early larval life.

All crosses were made in 60 ml bottles containing 20 ml of medium. Medium was of a cornmeal-agar-molasses-Karo-brewer's yeast type containing acid mix A of Lewis (LEWIS 1960) and sodium propionate to inhibit mold and, possibly, other micro-organisms. Paper strips of uniform size were added for extra pupation surface. Each individual cross was made with five flies of each sex per bottle; both sexes were aged separately for two days before mating. Each type of cross was replicated ten times. After a seven day egg laying period parents were transferred to a second bottle, for an identical period, and the data of the two weeks combined. Progeny were counted for eight days following first emergence. All crosses and all counts were made by the author. All work was done at $25 \pm 1^\circ\text{C}$.

RESULTS

The data, classified as to sex and phenotype and summed over all ten replicates, for each heterozygosity combination, are given in Table 2. No significant heterogeneity occurs among the replicates of any combination for either sex or phenotype, but statistically significant deviations from a 1:1 expectation occur with respect to both sex and phenotype within several of the combinations. When

TABLE 1
Crosses leading to the eight heterozygosity combinations in the progeny
SY indicates a Canton-S Y chromosome, WY a Wawawai Y chromosome; further explanation in text

1)	S/S;S/S;S/S	X S/SY;S/S;S/S°	→ S/S;S/S;S/S°	S/S;S/S;S/S,	S/SY;S/S;S/S°	S/SY;S/S;S/S
2)	S/S;S/S;S/S°	X X/WY;W/W;S/S	→ X/S;W/S;S/S°	X/S;W/S;S/S,	S/WY;W/S;S/S°	S/WY;W/S;S/S
3)	S/S;S/S;S/S	X S/SY;S/S;W/S°	→ S/S;S/S;S/S°	S/S;S/S;W/S,	S/SY;S/S;S/S°	S/SY;S/S;W/S
4)	X/X;W/W;S/S	X S/SY;S/S;W/S°	→ X/S;W/S;S/S°	X/S;W/S;W/S,	X/SY;W/S;S/S°	X/SY;W/S;W/S
5)	X/X;S/S;W/W	X S/SY;S/S;W/S°	→ X/S;S/S;W/S°	X/S;S/S;W/W,	X/SY;S/S;W/S°	X/SY;S/S;W/W
6)	W/W;W/W;W/W	X S/SY;S/S;W/S°	→ W/S;W/S;W/S°	W/S;W/S;W/W,	W/SY;W/S;W/S°	W/SY;W/S;W/W
7)	S/S;S/S;S/S°	X X/SY;S/S;W/W	→ X/S;S/S;W/S°	X/S;S/S;W/S,	S/SY;S/S;W/S°	S/SY;S/S;W/S
8)	W/W;W/W;W/W	X S/SY;S/S;S/S°	→ W/S;W/S;W/S°	W/S;W/S;W/S,	S/WY;W/S;W/S°	S/WY;W/S;W/S

TABLE 2

Summary of results for the eight heterozygosity combinations (#); percentages from pooled data

#	Flies counted					χ^2			Percent	
	♀ Drop	♀ Wild	♂ Drop	♂ Wild	Total	Sex	Phenotype	Contingency	Females	Drop
(1)	757	815	759	831	3162	0.10	5.34*	0.06	49.72	47.94
(2)	824	832	671	755	3082	17.16***	2.75	2.24	53.73	48.51
(3)	352	660	359	651	2022	<0.01	178.04***	0.13	50.05	35.16
(4)	725	784	691	812	3012	0.01	10.76**	1.30	50.10	47.01
(5)	660	680	648	664	2652	0.03	0.49	<0.01	50.53	49.32
(6)	765	837	706	742	3050	7.78**	3.82	0.31	52.52	48.23
(7)	686	752	627	638	2703	11.07**	2.19	0.93	53.20	48.58
(8)	804	786	757	821	3168	0.05	0.67	2.13	50.19	49.27
Total						36.47	204.06	7.10		
Total	5573	6146	5218	5914	22851	15.08***	70.47***	1.06	51.28	47.22
Heterogeneity χ^2						21.39**	133.59***	6.03		

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

the eight combinations are treated as groups, statistically significant heterogeneity exists with respect to sex ($\chi^2 = 21.4$, $P < 0.01$) and phenotype ($\chi^2 = 133.6$, $P < 0.001$). However, since contingency χ^2 values and their heterogeneity χ^2 imply that the distributions of sex and phenotype are independent, no further attention will be given to the sex data except to note that all statistically significant deviations are in the direction of increased female frequencies. By comparison, all statistically significant phenotype deviations are in the direction of reduced Drop frequencies. Despite the genetic dissimilarities of combinations 2, 5, 6, 7 and 8, Drop frequencies in these combinations are similar in that none falls significantly below 50% and none differs significantly from any other. All combinations are consistent in that all pooled frequencies fall below 50%, whether statistically significantly so or not.

The analysis of variance of Drop frequencies (Table 3) may be interpreted on the assumption that the eight heterozygosity combinations are exhaustive, or that they are a random sample of all possible comparable combinations (WRIGHT 1968). If F tests are made according to the former assumption, then the mean squares of all main factors and of all of their interactions are significant at $P < 0.001$, as shown. But if the tests are made according to the latter assumption, using SATTERTHWAITHE'S (1946) approximations for testing the main factors, then only the second order interaction is statistically significant, and this is significant at $P < 0.001$. Thus, while comparable experiments with other strains, or rearranged experiments with these strains, would in many cases be expected to yield results similar to those reported in this paper, not all could be expected to do so. Hence too broad a generalization is not justified.

The size and statistical significance of the interactions impose a limit on estimating the effects of heterozygosity on Drop frequency. An additional limitation results from the evident fact that the minimum heterozygosity effects of the S/S

TABLE 3

Analysis of variance, per culture, of Drop frequencies from the eight heterozygosity combinations, showing the effect of heterozygosity variation in the 3rd chromosomes of the Drop carriers (A), in the 3rd chromosomes of their wild-type competitors (B) and in the genetic backgrounds of both (C)

Source	DF	SS	MS	F
Total	79	0.220,106	—	—
A	1	0.038,152	0.038,152	45.3***
B	1	0.026,538	0.026,538	31.5***
C	1	0.016,558	0.016,558	19.7***
A × B	1	0.023,000	0.023,000	27.3***
B × C	1	0.023,820	0.023,820	28.3***
A × C	1	0.021,520	0.021,520	25.6***
A × B × C	1	0.009,922	0.009,922	11.8***
Residual	72	0.060,596	0.000,842	—

*** $P < 0.001$.

and W/W third chromosomes on viability are quite different, as will be shown. For these reasons the data are resembled for analysis as shown in Table 4.

Omitting combinations 5 and 6, because of the effect of the W/W third chromosomes, the data can be regrouped for analysis in three ways (Table 4). The first group, 3,4,7,8, compares the effect of heterozygosity variation in the third chromosomes of the Drop carriers (A) with that in the genetic background (C). Analysis of variance according to an exhaustive model shows that the mean squares of both factors and their interaction are significant at $P < 0.001$; under the random model only the interaction is statistically significant, and this at $P < 0.001$. The next group, 1,2,3,4, compares the third chromosome effects of the wild-type competitors of Drop (B) with the effects of the genetic background (C). Analysis of variance yields results which entirely parallel those of the previous group. The last group, 1,2,7,8, compares the effects of heterozygosity variation in the third chromosomes jointly (A,B) with that in the genetic background (C). Here, neither of the main factors nor their interaction is statistically significant under either model. The comparisons A–C and B–C provide a statistical parallel with the results of the variance analysis of Table 3, while the (A,B)–C comparison is quite different. This last is not unexpected since the A and B effects are approximately equal and are of opposite sign with respect to their effect on Drop frequency and since A and B are both low or both high in each of these four combinations. Lack of statistically significant interaction between (A,B) and C should not necessarily be taken as indicating a general lack of epistasis; lack of the former, here, simply means that the latter, if present, does not contribute to variation among the combinations and heterozygosity levels compared.

It would be interesting to have a better estimate of the effect of the A × B interaction since this interaction, which is between individuals of different genotypes, is fundamentally different from the A × C and B × C interactions, these latter being between third chromosomes and background within individuals.

TABLE 4
Mean percentage frequencies of Drop and their standard errors, for each of the eight combinations (#)

#	Mean $\pm$ SE	#	Mean $\pm$ SE	#	Mean $\pm$ SE	#	Mean $\pm$ SE
1	47.98 $\pm$ 0.90	3	35.27 $\pm$ 1.04	5	50.01 $\pm$ 1.10	7	48.54 $\pm$ 1.13
2	48.46 $\pm$ 0.78	4	47.11 $\pm$ 0.83	6	48.39 $\pm$ 0.73	8	49.36 $\pm$ 0.70

Analysis of variance, per culture, of Drop frequencies from all combinations except 5 and 6. Combinations analyzed shown by number, following mean squares. Other usages as in Table 3.							
MS (3,4,7,8)				MS (1,2,7,8)			
Total				Total			
A	0.0602***	Total (A,B)		Total		DF	
C	0.0400***	C		B		39	
A $\times$ C	0.0304***	(A,B) $\times$ C		C		1	
Residual	0.0009	Residual		B $\times$ C		1	
				Residual		1	
						36	

Simple effects of heterozygosis on Drop frequency, for each paired combination indicated.							
3,7	A (low C, high B)	= +13.27***	1,7	(A,B) (low C)	= +0.55	1,3	B (low C, low A)
4,8	A (high C, high B)	= + 2.25	2,8	(A,B) (high C)	= +0.90	2,4	B (high C, low A)
3,4	C (low A, high B)	= +11.84***	1,2	C (low (A,B))	= +0.48	1,2	C (low B, low A)
7,8	C (high A, high B)	= + 0.82	7,8	C (high (A,B))	= +0.82	3,4	C (high B, low A)
Interaction (A $\times$ C)	= - 5.51***		Interaction ((AB) $\times$ C)	= +0.17		Interaction (B $\times$ C)	= + 5.68***

*** P < 0.001.

TABLE 5

Comparison of combinations as to relative heterozygosity of phenotypic competitors and as to pooled percentage frequencies of Drop

For a given type and combination, in parentheses, relative heterozygosity is given as background:3rd chromosome and may be either low (L) or high (H) for a given category.

Combination	1	2	3	4	5	6	7	8
Drop type	(L:L)	(H:L)	(L:L)	(H:L)	(L:H)	(H:H)	(L:H)	(H:H)
Wild type	(L:L)	(H:L)	(L:H)	(H:H)	(L:L)	(H:L)	(L:H)	(H:H)
% Drop type	47.94*	48.51	35.16***	47.01**	49.32	48.33	48.58	49.27

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Tests of significance of the simple effects of Table 4 were made by t-test, the standard error of a difference between means being calculated as the square root of the sum of the relevant squared standard errors. Of the fifteen possible paired comparisons of combinations 1, 2, 3, 4, 7 and 8, none results in a statistically significant difference except those including combination 3, and these are all very highly significant ($P < 0.001$). These simple effects and their interactions clearly indicate that changes in heterozygosity in all three factors (A, B, C) can significantly alter Drop frequency, but only under certain genetic conditions. Such conditions can be more readily appreciated by considering next the single combinations shown in Table 5. The classification of categories of heterozygosity into "high" and "low" in this table ignores the possibly different contributions of X/S and W/S sex chromosomes in females as well as the difference between these and the sex chromosomes of the males. But attempts at refinement to more than two levels of heterozygosity have not, so far, made any difference in the conclusions. It is useful in this connection to summarize heterogeneity tests made with respect to all of 28 possible paired comparisons for the eight heterozygosity combinations, taken with respect to Drop frequency. Again it turns out that no paired combinations are significantly different except those involving combination 3, while all of the latter are significant at $P < 0.001$. Recall also that the Drop frequencies of combinations 1, 3 and 4 fall significantly below the 50% level and that no other Drop frequencies do.

Combination 1 is characterized by a significant reduction in Drop frequency. It is believed that this reduction results from the deleterious effect of the *Dr* gene, or at least of the *Dr* region, for the following reasons. *Dr* was transferred to a Canton-S background by repeated backcrossing and was maintained thereafter by mass culturing of the Drop flies. Therefore an *average* chromosome containing *Dr* and an *average* chromosome lacking it should differ only with respect to the genetic material in the near vicinity of the *Dr* locus. Also, combination 1 is a minimum heterozygosity combination, in the present context (Tables 1 and 5), with no difference in heterozygosity, on the average, between Drop carriers and wild-type flies other than in the *Dr* region, a condition evidently permitting *Dr* to have its maximum effect. By comparison, *Dr* is associated with a non-significant but consistent reduction in the frequency of Drop carriers in the remaining combina-

tions 2, 5, 6, 7 and 8, considered individually. But pooled, these combinations yield a Drop frequency of 48.78%, which is significantly below 50% ($\chi^2 = 8.79$, $df = 1$, $P < 0.01$).

In combination 2 the frequency of Drop carriers is intermediate between that of combination 1 and the theoretically expected value of 50% without being significantly different from either. A similar non-significant increase in Drop frequency is seen on comparing combinations 7 and 8, while a very highly significant increase appears when combinations 3 and 4 are compared. All of these increases are associated with an increase in the background heterozygosity of the segregant types.

Combination 3 gives the most striking result of any in the series—the increase in the third chromosome heterozygosity of the wild-type flies, compared with combination 1, is accompanied by a decline in the frequency of Drop carriers to 35.16%, a proportional decrease of nearly 30%. This decrease is characteristic of each replicate, the frequency of Drop ranging from 32% at lowest to 41% at highest. Here Drop frequency, as already indicated, differs significantly not only from the theoretical 50% value but from the Drop frequencies of all other combinations as well ($P < 0.001$). It is of interest that the difference between the total heterozygosities of the segregants is proportionally greater in combination 3 than in any other. In combination 4, with its very highly significant increase in Drop frequency, relative to 3, this proportionality has been diminished by the increase in background heterozygosity. In comparing 1,2 and 3,4 it may also be noted that in the latter pair the third chromosomes have a low heterozygosity in the Drop carriers and a high heterozygosity in their wild-type segregants, while in the former the third chromosome heterozygosities are low in both Drop and wild-type flies.

Combinations 5 and 6 might, on the basis of third chromosome heterozygosity, be expected to give results approximately the opposite of those of combinations 3 and 4, respectively, so far as Drop frequencies are concerned. Instead, Drop frequencies do not differ significantly from 50% in either combination. The wild-type flies of these two combinations have third chromosomes W/W, rather than S/S, as occurs in all other combinations in which the two members of a pair are from the same strain. Comparison of combinations 5 and 6 with combinations 7 and 8, as well as comparison of third chromosome pairs W/S^o and W/W within combinations 5 and 6, suggests that W/W is about equal to W/S as far as viability effects are concerned. Averaging the pooled frequencies of combinations 3 through 6 and classifying the third chromosome pairs as to strain and marker yields frequencies of 21% for S/S^o, 54% for W/S^o and W/S, and 26% for W/W, with respective viabilities of 76%, 100% and 95%, based on an expectation of 1:2:1. One result of such averaging is a slight diminution of the deleterious effect of *Dr* on the viability effect of S/W.

The increase in third chromosome heterozygosities of combination 7 over those of combination 1 does not significantly increase Drop frequency. In fact, combination 7 gives a result very much like that of combination 2, although third chromosome and background heterozygosities are reversed. Further, the widely dif-

ferent Drop frequencies of combinations 3 and 7 are seen to depend solely upon the different heterozygosities of the third chromosomes of the Drop carriers of these two combinations. Combination 8, characterized by high heterozygosity in the third chromosomes of both Drop and wild-type flies and in both of their backgrounds, has a Drop frequency slightly higher than that of combination 7. But this frequency, 49.27%, does not differ significantly from any other except that of combination 3, as already noted. But again, by comparison with combination 7, the increase is associated with an increase in background heterozygosity.

DISCUSSION

That the viability effect of S/W third chromosomes is nearly equal to that of W/W suggests that detrimentals in Canton-S chromosomes may be covered by less detrimental alleles in the Wawawai chromosomes. Since Canton-S, an old laboratory strain, may have accumulated detrimentals that would ostensibly be eliminated by selection in nature, Wawawai, a new laboratory strain, could be expected to have a lower proportion of such detrimentals and a higher proportion of both allelic and nonallelic detrimentals that are milder in their effect. One would also expect the old laboratory strain to be more homozygous than the new. Under these postulates, which might reasonably apply to many laboratory and natural populations (DOBZHANSKY 1948; WALLACE and KING 1952; ROBERTSON and REEVE 1955; CANNON 1963; SEIGER 1966; DOBZHANSKY and SPASSKY 1968), the effects of S/W and W/W chromosomes could be expected to be similar. The situation seems to correspond with one described by MUKAI and YAMAZAKI (1968) and MUKAI (1969a) in which accumulation of a greater than optimum number of spontaneous mutant polygenes, in *cis* arrangement, yielded a diminished degree of overdominance, unaffected by background heterozygosity. Thus other genetic influences than mass heterozygosity may be at work in the present situation. But aside from this, for the present, the following generalization may be drawn, letting A represent the third chromosome heterozygosity of the Drop carriers, B the third chromosome heterozygosity of the wild-type competitors of Drop, and C the background heterozygosity of both types, as before.

1) In each combination the *Dr* locus is consistently, though not always significantly, associated with a decrease in carrier viability. But appropriate pooling of certain combinations (2, 5, 6, 7, 8) shows the decrease to be a generally significant one. When C is increased this effect tends to be diminished; when B is increased the viability of Drop carriers is reduced further; when A is increased this last reduction may be largely offset. Discounting the deleterious effect associated with the *Dr* locus, it is seen that in any given combination the type with the higher total heterozygosity also has the higher frequency. Beyond this generalization further particulars may be drawn with respect to the effects on viability of variations in heterozygosity. These follow.

2) The maximal effects of A and B on viability are very nearly equal; the maximal effect of C is only slightly less. These results are, of course, not unexpected.

3) Viability is significantly changed only when there is a change in one or the other of A or B, and when C is low. If A and B change simultaneously and in the same direction there is no significant change in viability, irrespective of whether C is high or low.

4) Viability is significantly changed by a change in C only when coexisting A and B differ.

5) The effect of C is pronounced in some combinations and very slight in others. Its maximal effect occurs with low A and high B and would probably occur with high A and low B as well if the third chromosomes W/W and W/S were not so nearly alike in their effects. Its minimal effect occurs when A and B are both high or both low.

6) Statistical interaction is pronounced in some comparisons but nonsignificant in others. When A and B vary simultaneously and in the same direction interaction is not statistically significant. Statistically, very highly significant interactions occur when one of A or B varies with respect to variation in C. It is interesting that the two significant interactions, $A \times C$ and $B \times C$, are of opposite sign, depending on which comparisons are made. This raises a question as to whether the statistical interaction actually results from interchromosomal interaction in these data.

7) With respect to variability the standard errors of Table 4 show that an increase in third chromosome heterozygosity (A and/or B) increases variability when C is low (combinations 1, 3, 5, 7), but possibly has the opposite effect, or no effect, when C is high (combinations 2, 4, 6, 8). On the other hand, when paired standard errors are compared (1 vs 2, 3 vs 4, 5 vs 6, 7 vs 8) it becomes evident that an increase in C decreases variability, more when third chromosome heterozygosity is high, less when it is low. The net effect is that variability is decreased by an increase in heterozygosity and that this increase in heterozygosity tends to increase viability, when comparisons are made in this way. But perusal of both Tables 4 and 5 shows that in a more general sense the heterozygosity of a given type, without regard to its segregant, is not necessarily an indicator of its viability or of its variability.

8) Heterozygosity *per se*, possibly interchromosomal effects, and to some extent specific strain differences all contribute to the net level of viability.

It has of course been widely shown that an increase in heterozygosity increases the mean value of a trait (viability of *Drosophila melanogaster*: BONNIER 1961; BAND and IVES 1963; WALLACE 1963; DAWOOD 1969; TEMIN *et al.* 1969; fitness and fitness traits of *D. melanogaster*: CANNON 1963; KELLER and MITCHELL 1964; viability of *D. pseudoobscura*: DOBZHANSKY and LEVENE 1955; SPASSKY, DOBZHANSKY and ANDERSON 1965; fecundity and body size of *D. melanogaster*: ROBERTSON and REEVE 1955). The present data, with the exception of combinations 5 and 6, also show this direct relation between heterozygosity and viability within each combination. But when combinations are compared it is seen that it is the proportionally greater difference between the total heterozygosities of the segregants that counts for the greater viability of one of the segregant types. A somewhat similar dependence on the specificity of type has been shown by

WALLACE and KING (1952) and on the importance of coexisting types by LEWONTIN (1955) and DAWOOD (1969). WALLACE (1963) also reported that the average number of progeny per culture increased with increasing heterozygosity. In the present study the relation between total yields and proportional heterozygosity differences, as seen in combinations 1, 3, 4 and 8, is an inverse one. This probably results from the selective loss of Drop flies during preadult life.

It has also been widely demonstrated that an increase in heterozygosity decreases variability of a trait (viability of *D. melanogaster*: BAND and IVES 1963; DAWOOD 1969; fitness traits of *D. melanogaster*: KELLER and MITCHELL 1964; viability of *D. pseudoobscura*: DOBZHANSKY and LEVENE 1955; fecundity and body size of *D. melanogaster*: ROBERTSON and REEVE 1955). BAND and IVES (1963), reviewing a range of cases, further pointed out, with respect to viability, that such a decrease is the "usual situation observed regardless of species, auto-some, or method of making the test crosses." The present results agree with this except for the variation in Drop frequency seen on comparing combinations 1, 3, 5 and 7. Here the increase in heterozygosity within a given combination leads to an increase in the variability of Drop frequency. I am not acquainted with any other instance of this and have no explanation for it.

Studies of the effects of genetic background on viability (and other traits) have yielded exceedingly variable results. SEIGER's (1966) analysis showed extensive and varied effects of background with respect to male body weight in *D. melanogaster*; since all chromosome pairs were isogenic no heterozygosity was involved. Heterozygous viability effects of lethal-bearing second chromosomes from several natural populations of *D. pseudoobscura* were found, on the average, to be heterotic in native background and deleterious in foreign background (DOBZHANSKY and SPASSKY 1968; ANDERSON 1969). Corresponding effects of the second chromosomes from a natural population of *D. melanogaster* also proved to be deleterious when tests were made in a background of laboratory (foreign) chromosomes (HIRAIZUMI and CROW 1960; ANDERSON 1969). Superiority in the relative viability of the $+/e^{11}$ genotype of *D. melanogaster* (MOREE and KING 1961) was found to be diminished when background heterozygosity was increased (DAWOOD and STRICKBERGER 1964). MUKAI, YOSHIKAWA and SANO (1966) reported that in *D. melanogaster* both radiation-induced and newly arising spontaneous mutant polygenes affecting viability were mostly heterozygously overdominant in homozygous native background but became increasingly detrimental as background heterozygosity increased, more so with spontaneous mutants than with induced. With respect to overdominance, background heterozygosity was said to have a restrictive effect. These findings were later refined (MUKAI and YAMAZAKI 1968; MUKAI 1969a) by discovery of a *cis-trans* position effect. In homozygous background and with *cis* arrangement, overdominance increased with accumulation of mutant genes, up to a maximum of 11 mutants, after which it decreased; when these mutants were in *trans* arrangement their effect was highly deleterious. Increased background heterozygosity had no effect on the expression of overdominance when the number of mutants affecting overdominance was at an optimum, i.e., when it was from 8 to 11 per chromosome. TEMIN (1966) and KITAGAWA

(1967) reported that various categories of *D. melanogaster* mutants were usually heterozygously deleterious; KITAGAWA also stated that the deleterious effects were magnified in a foreign background, or in a homozygous background as compared with a heterozygous one. Similarly, TANO and BURDICK (1965) in studying female fecundity in *D. melanogaster* found that 6 out of 18 recessive lethals were heterozygously overdominant in homozygous background, while in heterozygous background they were slightly deleterious, though not statistically significantly so. Further examples simply add variety to this picture.

In the present study there appear to be two chief effects of genetic background. First, it appears to have a restrictive effect upon the deleteriousness associated with the *Dr* mutant. Second, it affects the viability of segregants to the extent that it diminishes or increases the proportional difference between the total heterozygosities of the segregants.

Results of studies on interchromosomal interaction have also yielded much variation. SPASSKY, DOBZHANSKY and ANDERSON (1965), using second and third chromosomes of *D. pseudoobscura* from six widely separated regions, reported a pronounced, positive interchromosomal synergism with respect to reduction of viability in double homozygotes. They pointed out, however, that such epistasis might not occur in all populations. TEMIN *et al.* (1969) in a similar experiment, but using second and third chromosomes of *D. melanogaster* from two localities in Madison, Wisconsin, reported a slight, positive interchromosomal epistasis which was not significantly different from zero. They pointed out that certain differences between their work and that of SPASSKY, DOBZHANSKY and ANDERSON might mean that the results were not significantly different. Other work with *D. melanogaster* tends to support this view. SEIGER (1966) found significant though irregular "isogenic" interchromosomal epistasis with respect to male body weight. KELLER and MITCHELL (1964) reported viability not to be affected by interchromosomal epistasis, though other fitness traits were. Summarizing, GOWEN (1952) indicated no epistasis between or among the three major chromosomes with respect to egg production. MUKAI (1969a) determined that the heterozygous viability effect of accumulated spontaneous mutant polygenes, in *cis* arrangement, in the second chromosome, was overdominant and was not affected by heterozygosity in the third chromosome. This relation was limited to second chromosomes with an optimum heterozygosity level, i.e., with 8–11 heterozygous loci. But KITAGAWA (1967) suggested that in his experiments a synergistic interaction between lethal genes and their genetic backgrounds had occurred. MUKAI (1969b) demonstrated a synergistic interaction with respect to viability among newly arising mutant polygenes located in the same chromosome and in homozygous condition; he suggested such interaction might be different from that between genes located in different chromosomes. ROBERTSON and REEVE (1955), basing their work on about ten inbred lines, noted that the occurrence of interchromosomal epistasis was widespread, though not invariable, and that it depended on degree of heterozygosity, background, strain, relative magnitude of the trait, and specificity of genotypic combination, with respect to the two traits, egg production and body size. Because of epistasis, variations in heterozy-

gosity could not account for all trait variations. They further noted that egg production and body size, despite their being somewhat different traits, presented "the same picture in terms of the nature of the genetic interaction."

In toto, the results of others and of the present work suggest that in the present data statistical interaction may not represent interchromosomal interaction. But comparisons for simple and interaction effects such as those given in Table 4, made not with Drop frequencies but with the proportional heterozygosity differences suggested by Table 5, do not change the essential results. Therefore it appears that in the present study proportional heterozygosity differences underlie the expression of interchromosomal epistasis, or at least contribute to it. (A proportional heterozygosity difference is estimated by the difference between the frequencies of Drop and wild-type flies in any given combination.)

None of the crosses in these experiments yields S^*/S^* . But since lethality of S^*/S^* takes place largely during early larval life it seems reasonable to regard the present results as if the three marker genotypes, S^*/S^* , $S^*/+$ and $+/+$ had developed together in the same cultures. But then the typical criterion for heterosis, $S^*/S^* < S^*/+ > +/+$, would not be realized in any of the present experiments since the third chromosomes S/W and W/W have about equal effects on viability and since $S/W > S^*/S$. However, the postulated genetic basis of the present results entirely satisfies the conventional criterion when *Dr* is transferred to a Wawawai chromosome, for this makes possible the three marker classes with, according to recent data, the relations $W^*/W^* < W^*/S > S/S$, at least when background heterozygosity is low. So the present results may be interpreted on the basis of a heterosis mechanism comparable to the one studied by CARSON (1958, 1961), SUSMAN and CARSON (1958), SMATHERS (1961) and CANNON (1963).

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