THE GENETIC VARIANCE FOR VIABILITY AND ITS COMPONENTS IN A LOCAL POPULATION OF *DROSOPHILA MELANOGASTER*[1]

TERUMI MUKAI, RICARDO A. CARDELLINO, TAKAO K. WATANABE[2] AND JAMES F. CROW

Department of Genetics, North Carolina State University
Raleigh, North Carolina 27607

and

University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT

Two hundred and ninety second chromosomes extracted from a natural population of *Drosophila melanogaster* were analyzed to estimate the genetic variance of viability and its components by means of a partial diallel cross (Design II of COMSTOCK and ROBINSON 1952). The additive and dominance variances are estimated to be 0.009 and 0.0012. Using the dominance variance and the inbreeding depression, the *effective* number of overdominant loci contributing to the variance in viability is estimated to be very small, a dozen or less. Either the actual number of loci is small, or the distribution of viabilities is strongly skewed with a large majority of very weakly selected loci. The additive variance in viability appears to be too large to be accounted for by recurrent harmful mutants or by overdominant loci at equilibrium with various genetic parameters estimated independently. The excess might be due to frequency-dependent selection, to negative correlations between viability and fertility, or possibly to the presence of a mutator. The selection for viability and fertility, or possibly to the presence of a mutator. The selection for viability at the average polymorphic locus must be very slight, of the order of 10^{-3} or less.

WE present here the results of a partitioning of the genetic variance for viability into additive and dominance components and an attempt to determine the relative contributions of different kinds of genes to these components. The discovery of numerous polymorphisms for isozymes and other proteins (LEWONTIN and HUBBY 1966) has added to the interest in ways by which the population variability is maintained. MUKAI and MARUYAMA (1971) have suggested that these polymorphisms are mainly neutral, or nearly so, because in simulation studies based on empirically estimated population parameters they could account for the homozygous genetic load in *D. melanogaster* by assuming only ten to twenty heterozygous loci per second chromosome. In this paper we

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[2] Present address: National Institute of Genetics, Mishima, Japan.

give evidence that the dominance variance in viability, insofar as this is caused by overdominant genes, is either due to a very small number of loci, or the average effect on viability per locus is very small. The data also suggest that the additive variance is too large to be accounted for by recurring harmful mutants or overdominant loci at equilibrium.

EXPERIMENTAL PROCEDURES

The chromosomes used in the experiments were descended from 691 second chromosomes extracted from a population of *Drosophila melanogaster* in Reedy Creek State Park near Raleigh, North Carolina. The method of isolating chromosomes and maintaining them in stock cultures is shown in Figure 1. Each chromosome was maintained in a stock heterozygous with the second-chromosome balancer *SM1*. This chromosome can be identified by the dominant curly wing phenotype, abbreviated *Cy*. *Cy/Cy* homozygotes are lethal. The salivary gland chromosomes of each line were examined by MR. OSAMU YAMAGUCHI; 130 of the 691 were found to carry inversions.

Experiment 1: 154 chromosome lines were randomly chosen and divided into 11 groups of 14 lines each. Each group was divided into two equal subgroups, and each line of the first subgroup was mated with each of the second to constitute a 7×7 partial diallel arrangement with 7 rows and columns. From the ith line (row) 5 $Cy/+_i$ females were mated to 5 $Cy/+_j$ males from the jth line (column)

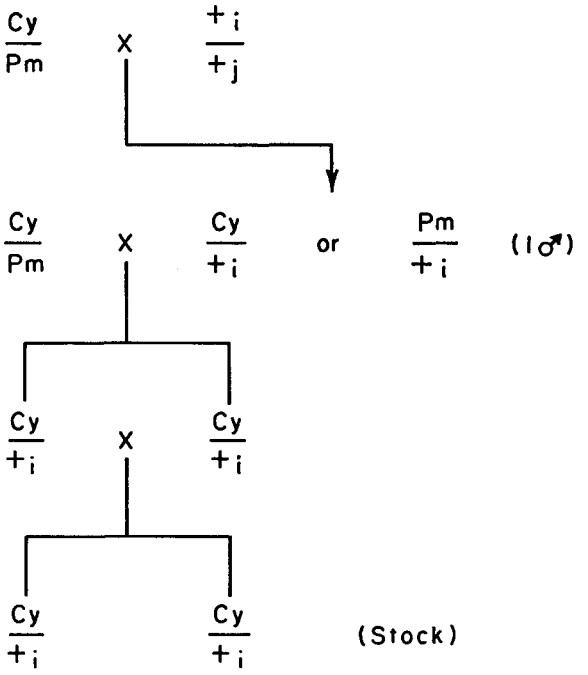

FIGURE 1.—The mating system used to establish stocks.

in two replicates. Reciprocal crosses, $Cy/+_j$ females $\times$ $Cy/+_i$ males, were also made, again in two replicates. The design is equivalent to Design II of COMSTOCK and ROBINSON (1952) with reciprocal matings.

Four days after crosses were made, the flies were transferred to a second vial, and after four more days all the parents were discarded. The offspring counts were continued until the eighteenth day after the matings (or transfers) were made. The counts from the original and transfer vial were pooled. The analysis was done in two ways. In the first, the viability was expressed as a ratio, (Number of wild-type flies)/(Number of $Cy + 1$), the 1 being to correct a bias from averaging ratios (HALDANE 1956). Later, each viability ratio was standardized by the average viability of the group. Thus the average viability ratio of the population is necessarily 1.00. In the second analysis that relative viability was expressed as the logarithm of the ratio of wild-type to Curly flies. In group 10 one line was missing by accident. The number of matings was therefore $[10(7 \times 7) + (7 \times 6)] \times 2$ reciprocal crosses $\times$ 2 replicates = 2128, or 4256 vials counted.

Of the 153 chromosomes tested, 19 carried inversions.

Experiment 2: This was done exactly the same as Experiment 1, except that none of the chromosomes had an inversion. One hundred and forty chromosomes, different from those of Experiment 1, were chosen at random. Three were lost in the course of the Experiment. The total number of matings was 1876, or 3752 vials counted. However, one cross (in group 8) was lethal due to allelism of lethal genes carried by the parental chromosomes. Thus, the total number of matings available was 1872 or 3744 vials counted.

Experiment 1 was begun approximately 2 months after the flies were captured. Experiment 2 was begun approximately a year later. Except during the experiments, the flies were kept at 19°.

ANALYSIS OF VARIANCE

The total sum of squares in each group was partitioned into Rows, Columns, Row $\times$ Column Interaction, and Error, considering reciprocal crosses as additional replicates (see DATA AND ANALYSIS below). The sums of squares of all the groups were pooled and the variance components were estimated from the pooled data.

The variance components were estimated as follows:

Source of variation	Degrees of freedom	Mean squares	Expectation of mean squares
Females (Rows)	$\sum_{i=1}^{m} (a_i - 1)$	MS_R	$V_E + nV_{RxC} + k_1V_R$
Males (Columns)	$\sum_{i=1}^{m} (b_i - 1)$	MS_C	$V_E + nV_{RxC} + k_2V_C$
Interaction	$\sum_{i=1}^{m} (a_i - 1)(b_i - 1)$	MS_{RxC}	$V_E + nV_{RxC}$
Error	$\sum_{i=1}^{m} a_ib_i(n - 1)$	MS_E	V_E

where a_i is the number of female parents in group i ($i = 1, \ldots, m$), b_i is the number of male parents, m is the number of groups, and n is the number of replicates per cell. When there were no missing cells, k_1 was calculated by $\Sigma nb_i(a_i - 1)/\Sigma(a_i - 1)$ and k_2 by $\Sigma na_i(b_i - 1)/\Sigma(b_i - 1)$. When there were missing cells, quantities corresponding to na_i or nb_i were calculated by the least-squares method (cf. SCHAFFER and USANIS 1969), and after that the same procedure as above was used. Except when a line was missing, $a_i = b_i = 7$, and $n = 4$ for all cells, and $k_1 = k_2 = 28$.

For the groups having missing cells (about which detailed description will be given later), the computer program written by SCHAFFER and USANIS (1969) was employed for the analysis of variance.

Since V_C and V_R are expected to be the same, their pooled estimate ($\hat{V}_P$) was obtained for the estimation of the additive variance.

$$\hat{V}_P = \frac{\dfrac{MS_R\Sigma(a_i - 1) + MS_C\Sigma(b_i - 1)}{\Sigma(a_i - 1) + \Sigma(b_i - 1)} - MS_{RxC}}{\dfrac{k_1\Sigma(a_i - 1) + k_2\Sigma(b_i - 1)}{\Sigma(a_i - 1) + \Sigma(b_i - 1)}}$$

The same *Cy* chromosome was used for all matings. The progeny within a cell are full sibs as regards the second chromosome and, therefore, all the wild-type progeny have identical second chromosomes. This means that the variance of the standardized viability ratios among cells is the total genetic variance, which is $V_A + V_D + V_{AA} + V_{AD} + V_{DD}$ plus higher order epistatic terms, where V_A is the additive (genic) variance, V_D is the dominance variance, and the double subscripts indicate epistatic combinations of these (KEMPTHORNE 1957). Within a row or column the progenies in different cells are half-sibs with respect to the second chromosomes. Since the parents in a line have identical second chromosomes, the half-sib covariance of the viability ratios is equivalent to that from homozygous parents, or $1/2\,V_A + 1/4\,V_{AA}$. The total variance among cells (matings) is $V_R + V_C + V_{RxC}$. Since $V_R = V_C = 1/2\,V_A + 1/4\,V_{AA}$ and $V_R + V_C + V_{RxC} = V_A + V_D + V_{AA} + V_{AD} + V_{DD}$, we have the estimating equations

$$V_A + 1/2\,V_{AA} = 2V_P = V_R + V_C \tag{1}$$

$$V_D + 1/2\,V_{AA} + V_{AD} + V_{DD} = V_{RxC}\,. \tag{2}$$

The other chromosomes, *X*, *Y*, *III*, and *IV*, are not controlled in these experiments. Variance components from these sources will affect the results only if they differentially influence the viability of wild-type and Curly flies. However, interchromosomal epistasis is probably small for chromosomes of near normal viability, so this source of variability has been ignored. We shall also ignore intra-chromosome epistasis in the subsequent analysis.

However, this simple analysis must be modified because the same chromosomes appear in both the +/+ and *Cy*/+ classes and introduce correlations between the numerator and denominator of the viability ratio. This can be taken into account by the following procedure, which is very rough but accurate

enough for our purpose. Let s_i be the additive deviation of genes on the ith chromosomes, s_c the deviation of the Cy chromosome, d_{ij} be the dominance deviation of genes in the $+_i/+_j$ heterozygote, and d_{ic} be the dominance deviation of genes in the $Cy/+_i$ heterozygote (these are all assumed to be small quantities with mean 0). Recall that the mean ratio of $+/+$ to $Cy/+$ flies has been standardized to equal one. Then the expected standardized ratio of wild-type to Curly flies in the progeny of the $Cy/+_i \times Cy/+_j$ mating, which consist of $+_i/+_j$, $Cy/+_i$, and $Cy/+_j$ genotypes, is,

$$\frac{1 + s_i + s_j + d_{ij}}{\tfrac{1}{2}(1 + s_i + s_c + d_{ic}) + 1/2(1 + s_j + s_c + d_{jc})} .$$

If the s and d values are small, this is approximately equal to

$$1 + (\tfrac{1}{2})(s_i + s_j) - \tfrac{1}{2}(d_{ic} + d_{jc}) - s_c + d_{ij} ,$$

If all epistasis is ignored, the variance of the row effects plus that of the column effects is

$$V_R + V_C = \tfrac{1}{4} V_A + \tfrac{1}{2} V_D .$$

There is no variance contribution from s_c since all Curly chromosomes are the same. The interaction variance is

$$V_{R\times C} = V_D ,$$

again ignoring epistasis.

The equations for estimating V_A and V_D become

$$V_A = 4(V_R + V_C - 1/2\, V_{R\times C}) \tag{3}$$

and

$$V_D = V_{R\times C} . \tag{4}$$

DATA AND ANALYSIS

An analysis showed no significant differences between reciprocal crosses in Experiment 1 ($F_{532,1064} = 1.028$). However, significant reciprocal differences were detected in Experiment 2 ($F_{463,926} = 1.28$, $P < 0.01$), but these differences were due almost entirely to groups 8 and 10. The reciprocal differences of these outlying groups were traced for several additional generations. This was possible because the chromosomes of Experiment 2 were analyzed again after 10, 20, and 30 more generations maintained through heterozygous males (CARDELLINO and MUKAI 1975). However, no consistency in reciprocal differences among generations was found. Thus, it was concluded that the reciprocal differsences detected in groups 8 and 10 in Experiment 2 were not due to meiotic drive or maternal effects (the cytoplasm of all lines were the same), but to unknown experimental errors in these crosses. In fact, the F value due to reciprocal differences at generation 30 was 1.073 ($df = 410, 820$; $P >> 0.05$). Therefore, the sum of squares due to reciprocal differences was not separated, but 4 extreme cells in group 8 and 7 in group 10 were removed from the analysis. After removal of these outliers, F turned out to be 1.157 ($df = 457, 914$; $P \approx 0.05$). The total number of matings utilized in Experiment 2 became 1828 or 3656 vials counted.

TABLE 1

Analysis of variance of viability data
Experiment 1. 153 chromosomes of which 19 carried inversions

Source	*D.F.*	*M.S.*	*F*
Parents ($R + C$)	131	0.065034	2.75***
Interaction ($R \times C$)	390	0.023663	1.24**
Error	1596	0.019072	
Total	2117		

Experiment 2. 137 chromosomes, all inversion-free

Source	*D.F.*	*M.S.*	*F*
Parents ($R + C$)	117	0.060083	2.79***
Interaction ($R \times C$)	330	0.025428	1.18*
Error	1371	0.021542	
Total	1818		

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

The overall analysis of variance for the two experiments is shown in Table 1. The sums of squares for rows and columns are combined, as are their degrees of freedom. From the values in Table 1 the components of genetic variance and their standard errors were computed from Equations 1 and 2 (see TALLIS 1959). These are shown in Table 2. As can be seen, there is no appreciable difference when the 19 chromosomes carrying inversions are omitted, so there is no evidence from these experiments that the inversions have important effects on the genetic variance for viability and its components. Furthermore, there is very little difference in Experiments 1 and 2 despite the stocks in Experiment 2 having been kept one year at 19° before the experiments was done.

At the bottom of Table 2 is the second analysis, based on the logarithm of the ratio of wild-type to Curly flies in each culture. The final pooled estimate was

TABLE 2

Estimated variance components for viability

	$V_R + V_C$	$V_{R \times C}$
Experiment 1 Including inversions	0.002974 ± 0.000590	0.001148 ± 0.000456
Excluding inversions	0.002999 ± 0.000676	0.001092 ± 0.000531
Experiment 2	0.002599 ± 0.000608	0.000971 ± 0.000536
Logarithmic transformation		
Experiment 1	0.003184 ± 0.000632	0.001350 ± 0.000486
Experiment 2	0.002628 ± 0.000650	0.000991 ± 0.000612
Pooled	0.002914 ± .000453	0.001211 ± 0.000381

arrived at by weighting the two experiments inversely by their error variances.

V_A as estimated from Equation 3 is 0.00923 for the pooled, logarithmically transformed data, as compared to 0.00291 for $V_R + V_C$ in Table 2. For the untransformed ratios, the estimate of V_A is .00960.

WALLACE and DOBZHANSKY (1962) have reported that the Curly chromosome is almost completely dominant to its homolog. In that case V_A should be estimated simply by $V_R + V_C$, rather than by Equation 3. The estimate of 0.009 may then be too high. It is unlikely, however, that a particular chromosome can influence the genetic variance among all the genes on its homologs. Furthermore, recent experiments (MUKAI, unpublished) show normal variance among the chromosomes heterozygous with *Cy*. We therefore think that Equation 3 provides the best estimate for the additive variance.

INTERPRETATION OF VARIANCE COMPONENTS

The parameters for a locus with two alleles in Hardy-Weinberg proportions are as follows:

Genotype	AA	Aa	aa	
Frequency	p^2	$2pq$	q^2	(5)
Relative viability	1	$1 - hs$	$1 - s$	

With this model, the total genetic variance for a single locus (V_t), the additive (genic) component (V_a), and the dominance component (V_d) are given by:

$$V_t = pqs^2[2(1 - 2pq)h^2 - 4q^2h + q(1 + q)] \quad (6)$$

$$V_a = 2pqs^2[(p - q)h + q]^2 \quad (7)$$

$$V_d = p^2q^2s^2(1 - 2h)^2 \ . \quad (8)$$

From the relations (5) the mean viability, $\bar{w}$, of a randomly mating population is $1 - 2pqhs - q^2s$ and that of a completely inbred population is $1 - qs$. Therefore, the inbreeding decline from $F = 0$ to $F = 1$ is

$$\begin{aligned} l &= (qs - 2pqhs - q^2s)/\bar{w} \\ &= [pqs(1 - 2h)]/\bar{w} \ . \end{aligned} \quad (9)$$

This is the inbred load for viability, with the convention that the inbred viability is measured as a deviation from a randomly mating population, rather than from the most viable genotype as conventional load theory prescribes (e.g., MORTON, CROW and MULLER 1956; CROW 1970). Note that $V_d = l^2$ when $\bar{w} = 1$.

Assuming that the effects at individual loci on the chromosome are small enough and/or nearly enough to being independent to be additive, the inbreeding decline for the whole chromosome is

$$L = \Sigma l = n\bar{l} \quad (10)$$

where n is the number of loci. Likewise under the same assumptions the dominance variance for the whole chromosome is

$$V_D = \Sigma V_d = n\overline{V_d} \ . \quad (11)$$

Our viability ratios are standardized to a mean value of 1, which means that s in Equations (6)–(8) is equivalent to $s/\bar{w}$ in (9). Therefore $V_d = l^2$, and

$$V_D = \Sigma l^2 = n(l^2 + V_i) \ . \tag{12}$$

Therefore, the ratio L^2/V_D provides a minimum estimate of the number of relevant loci.

Note that the foregoing equations require no assumption that the gene frequencies are at equilibrium. Dr. Joseph Felsenstein (personal communication) noted earlier that for an overdominant locus with two alleles at gene frequency equilibrium the square of the inbreeding decline is equal to the genetic variance, and that this might be used as a way of estimating the gene number associated with a quantitative trait. We now see that his relationship is a consequence of (12), because at equilibrium with overdominance all the variance is in the dominance component.

The additional quantities needed to interpret these variance components come from other studies. The total viability load due to new mutations, $\Sigma u_i s_i$, is estimated to be 0.0040 ± 0.0002 (Mukai 1964; Mukai *et al.* 1972). This may be written $n\bar{u}\bar{s}$, where n is the number of loci, $\bar{u} = (\Sigma u)/n$ is the average mutation rate per locus, and $\bar{s} = (\Sigma us)/\Sigma u$ is the average effect of a mutant on viability weighted by its mutation rate. The number of loci is taken as 2500. This is based on the number of salivary chromosome bands, an assumption made plausible by the correspondence between the number of bands and the number of complementation units in an intensively studied region of the Drosophila X chromosome (Judd, Shen and Kaufman 1972). Σu was estimated as greater than 0.17 and $\bar{s}$ as less than 0.023, but it should be realized that the product of these two quantities, .0040, is known with much more precision than either factor (see Mukai *et al.* 1972).

The average dominance of newly arisen viability mutants is estimated to be about 0.4 (Mukai 1969), and for those in a natural population about half this value, 0.2. Finally, the homozygous detrimental load (Greenberg and Crow 1960), expressed as a deviation from the average viability of a randomly mating population, is estimated to be 0.33 in this population (Mukai and Yamaguchi 1974).

In the following calculations we shall take the basic parameters to be:

Load due to new mutations:	$\Sigma us = n\bar{u}\bar{s} = 0.004$
Additive variance:	$V_A = 0.009$
Dominance variance:	$V_D = 0.0012$
Average homozygous selective disadvantage:	$\bar{s} = 0.02$
Mutation rate per locus:	$u = 0.00008$
Homozygous detrimental load:	$L = 0.3$
Average dominance of mildly deleterious mutants in equilibrium population:	$\bar{h} = 0.2$
Number of loci per second chromosome:	$n = 2500$

THE EFFECTIVE NUMBER OF LOCI CONTRIBUTING TO THE DOMINANCE VARIANCE

We emphasize that Equations 6–12 do not require any assumption that the gene frequencies are in equilibrium. We shall use Equations 10 and 12 to estimate the number of loci contributing to the dominance variance. We define n_e as the effective number of loci. This will be the actual number when l is the same for each locus; otherwise it is a minimum estimate. If V_l is taken as zero, the effective number of loci is estimated by L^2/V_D.

Taking the values above, the effective number of loci contributing to the dominance variance is $(0.3)^2/0.0012 = 75$. Of course, if the loci contribute very unequally to the variance the actual number may be much larger.

Further analysis requires assumptions about equilibrium gene frequencies.

RECESSIVE MUTANTS

Letting $h = 0$, the equilibrium frequency of the recessive mutant gene is

$$q = \sqrt{u/sc} \tag{13}$$

where c is a quantity introduced to relate selection on total fitness to that on viability alone. Presumably, $c > 1$.

Substituting (13) into (8) and (11) and neglecting terms of order q^3

$$V_D \approx \Sigma(us/c) = \left(\frac{1}{c}\right) \Sigma us \tag{14}$$

if c and us are independent. If $\Sigma us = .004$ and the observed value of V_D is .0012, this implies that c is 3.33; that is to say, selection on total fitness is about 3 times as great as that on viability alone. If c is variable, as it surely must be, the smaller values of c are most influential, since it is the harmonic mean that is relevant.

On the other hand, if $s = .02$, $u = 8 \times 10^{-5}$, $c = 3.3$, and $n = 2500$, the expected value of L, from (13), (9), and (10) is 1.7. The observed inbred load, L, is 0.3 and this surely includes effects other than recessive genes. We conclude, in agreement with other studies (e.g. MUKAI *et al.* 1972), that most viability mutants are not fully recessive. Consequently, we suspect that, although there may well be completely recessive mutants in the population, they do not account for all the dominance variance.

PARTIALLY DOMINANT MUTANTS

If there is partial dominance $(0 < h < 1)$ the equilibrium gene frequency is

$$q \approx u/chs \tag{15}$$

where u is the mutation rate from A to a. We assume that $h^2 >> u/cs$ and $cs >> u$. Then, substituting (15) into (7) and (8), we obtain

$$V_a \approx 2uhs/c \tag{16}$$

$$V_d \approx [u(1-2h)/ch]^2 \ . \tag{17}$$

Their ratio is

$$R = \frac{V_d}{V_a} \approx \frac{u(1-2h)^2}{2h^3cs} \quad . \tag{18}$$

This ratio decreases as h increases toward 1/2 and for this value all the variance is additive.

If $u = 8 \times 10^{-5}$, $s = .02$, and $h = .4$, $R = .0013$ if $c = 1$ and is less if $c > 1$. If $h = .2$, $R = .09$ if $c = 1$ and is less if $c > 1$. Since the observed value of V_D is .0012 and V_A is .009, these calculations show that the dominance variance is too large relative to the additive variance to be accounted for by mutants with $h > .2$.

We cannot be sure of this argument, though, because the quantities in (18) are certainly not constant. R is likely to be increased if h is variable because of the influence of small values of h entering as h^3 in the denominator. Furthermore, u may be underestimated. So, although the high observed values of h in other experiments argue that V_D is not entirely accounted for by partially dominant loci, we cannot rule out this possibility from our data.

On the other hand, the inbreeding decline, L, is easily accounted for by partially dominant mutants. $L = nu(1-2h)/ch$. If $u = 8 \times 10^{-5}$, $n = 2500$, $c = 2$, and $h = 0.2$, then L agrees with the observed value, 0.3.

OVERDOMINANT LOCI

In view of the analysis above it is likely, although by no means proven, that not all the dominance variance is caused by recessive and partially dominant mutants. An obvious candidate for the remaining variance is overdominant loci.

We consider a somewhat restricted model in which all heterozygotes are of equal viability (say, 1), but homozygotes may differ. The homozygote A_iA_i has viability $1 - s_i$. We are assuming that mutation is small enough to play no significant role in determination of equilibrium frequencies and that all selection is on viability.

The mean viability at equilibrium is

$$\bar{w} = 1 - \Sigma s_i p_i^2 = 1 - p_i s_i = 1 - \frac{\tilde{s}}{k} \tag{19}$$

where $\tilde{s}$ is the harmonic mean of the s_i's and k is the number of segregating alleles at this locus (see CROW and KIMURA 1970, pp. 304–308). The mean viability of homozygotes is $1 - \tilde{s}$. Measured from the population mean, the inbred viability load is

$$L = \tilde{s} - \frac{\tilde{s}}{k} = \tilde{s}\,\frac{k-1}{k} \quad . \tag{20}$$

The variance, which at equilibrium is all dominance variance, is

$$V_d = \Sigma p_i^2 s_i^2 - (\Sigma p_i^2 s_i)^2 = \tilde{s}^2\,\frac{k-1}{k^2} \quad . \tag{21}$$

Summing over loci

$$L = \Sigma \tilde{s}_j (k_j - 1)/k_j = n\,\overline{[\tilde{s}(k-1)/k]} \tag{22}$$

$$V_D = \Sigma \bar{s}_j^2 (k_j - 1)/k_j^2 = n\,\overline{[\bar{s}^2(k-1)/k^2]} \quad . \tag{23}$$

We can then estimate the effective number of loci by

$$n_e = \frac{L^2}{V_D(k-1)} \tag{24}$$

where n_e is a correct estimate of n if all loci act equally, but is an underestimate otherwise. The observed dominance variance is 0.0012. Suppose that 0.001 is the contribution from overdominant loci. The inbred load from overdominance is not likely to be greater than 0.1; the maximum possible, if the entire inbred load has this cause, is 0.33. Here are a few representative numbers:

V_D	L	k	n_e
.001	.3	2	90
	.1	2	10
	.3	4	30
	.1	4	3 .

The situation is more complicated if the genes that affect viability also have effects on other aspects of fitness, such as fertility. However, the equilibrium frequency of the alleles depends only on their relative homozygous disadvantages, not their absolute values. If the decrease in fitness of a homozygote is proportional to the decrease in viability, Equations 19–24 are still correct for viability.

This is a dubious assumption, however. The calculations in the paragraph above for $k = 2$ do not depend on gene frequency equilibrium, as mentioned earlier. We suspect, but cannot prove, that when $k > 2$ the effective number of loci becomes smaller for nonequilibrium conditions as it does for our equilibrium model.

It thus appears that if the typical segregating overdominant locus has more than two alleles, the effective number of such loci must be quite small—a dozen or less per chromosome, in agreement with the analysis of MUKAI and MARUYAMA (1971). Of course, if the loci differ greatly in the magnitude of their effects the actual number may be much larger than the effective number. In particular, this analysis does not rule out a small number of loci with relatively large effects and a much larger number with near-zero effect on viability.

The proportion of segregating loci has been estimated from electrophoretic data to be as high as 50% and the average heterozygosity about 20% in Drosophila populations (O'BRIEN and MACINTYRE 1969). This would imply something like 1000 segregating loci on the second chromosome. If these are all overdominant, we can use Equation (23) to estimate $\bar{s}$. Taking $V_D = .001$, $n = 1000$, and $k = 5$, this gives $\bar{s} = 0.0025$. If $L = 0.1$, $n = 1000$, and $k = 5$, $\bar{s}$ becomes 0.00013 from (22). To the extent that these loci are maintained by selection for viability, the amount of selection must be very small, of the order 10^{-3} or less.

THE ADDITIVE VARIANCE

There remains the question of what accounts for the additive variance for viability, estimated as 0.009.

For recessive mutants with the parameters that we have been using ($u = 8 \times 10^{-5}$, $s = .02$, $h = 0$, $c = 2$, and $n = 2500$), the additive variance for viability from (13) and (7) is roughly 10^{-4}, much smaller than the observed value. For partially dominant loci, the situation is about the same. Even if h is as high as 0.4, the expected additive variance from (16) is about 0.0016, again too small for the observed value. It is possible that u and s are incorrectly estimated, but the experimental procedures (MUKAI *et al.* 1972) are such that the product us is estimated much more accurately than either component; these two terms enter together in (16). So it would appear that recessive and partially recessive viability mutants are not sufficient to account for the observed additive variance. Neither are overdominant loci if the population is at equilibrium, for the additive variance should be zero.

What accounts for the large additive variance for viability? One possibility is a mutator gene. It is known that chromosomal aberrations and associated lethal mutations occur at a high frequency in this population (CARDELLINO and MUKAI, 1975; YAMAGUCHI, CARDELLINO and MUKAI, in preparation). However, there is no evidence as yet that the mutator produces polygenic mutations. Any effect on the additive variance would have to be mainly through the heterozygous effects of lethals and chromosome aberrations.

A second possibility is that the genes are not at the same equilibrium values as if these were determined solely by viability differences. Total fitness also includes components not measured in our experiments, in particular fertility. There is evidence for negative correlations between viability and fertility for high viability chromosomes (cf. HIRAIZUMI 1961). This could lead to a high additive variance for viability even though the variance for total fitness is restricted.

A third possibility is frequency-dependent selection. If there are a number of viability genes with intermediate heterozygotes ($h \approx 0.5$), but which are frequency-dependent, they might make a substantial contribution to the additive variance in these experiments. Each gene in these experiments has a frequency 1 or 1/3, which is probably not its equilibrium value in nature.

DISCUSSION

All our calculations have assumed that the population is at or very near equilibrium. There are several reasons for thinking that this is a reasonable assumption. One is that the parameters of this population resemble very closely a large population cage that has been maintained in the laboratory for many years. Furthermore, these parameters (lethal frequency, inbred load, allelism of lethals, isozyme frequencies) are quite stable from year to year. These points have been discussed in more detail by MUKAI and YAMAGUCHI (1974).

We have also not taken epistasis into account. Studies of chromosomes taken from natural populations and made homozygous (TEMIN *et al.* 1969) and of newly accumulated viability mutants (MUKAI 1969) both show evidence of slight synergism for mildly deleterious effects on viability. However, the magnitude of this epistasis is small, especially in high viability chromosomes. These experiments do not, however, rule out the possibility of positive and negative epistasis that are approximately canceling as regards mean effects, but which can still contribute to the variance.

In addition to the assumptions regarding equilibrium and non-epistasis, there is the problem that laboratory experiments cannot duplicate the complexity and diversity of natural habitats, which may greatly enhance the expression of genetic variability. Our actual measurements are on viability rather than total fitness.

The two experimentally determined quantities measured from chromosomes extracted from natural populations are the inbreeding decline and the genetic variance, the latter divided into additive and dominance components. Although, for the reasons given above, we cannot regard our conclusions as proven, the inbreeding decline in viability is easily accounted for by recessive or partially dominant mutants occurring at rates consistent with laboratory data. On the other hand, the genetic variance of the population appears to be too large to be accounted for by such mutants and therefore, we believe, is caused by loci with some form of balancing selection, such as overdominant or frequency-dependent selection, by negative correlations between viability and fertility, and possibly by the effects of a mutator.

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Corresponding editor: T. PROUT