

# SYNERGISTIC EFFECT OF INBREEDING ON VIABILITY IN *DROSOPHILA VIRILIS*

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## ABSTRACT

The effect of inbreeding on egg to adult viability was determined for *Drosophila virilis* over a wide range of inbreeding levels ( $0 < F < .734$ ). The quantity  $-\log_e$  (Viability) was found to be a curvilinear function of  $F$ , indicating synergistic interaction among loci. The curvature was not evident, however, below  $F = .500$ . The values of  $A$  and  $B$  (MORTON *et al.* 1956) were calculated to be .06 and .77-.86, respectively. This extremely small value of  $A$  yielded a very large value for the  $B/A$  ratio i.e., 12.51-14.99.

TWO ingeneous methods have been developed for the estimation of the genetic load: one by GREENBERG and CROW (1960) and the other by MORTON, CROW and MULLER (1956). In the former method, the amount of genetic load can be directly measured by homozygous viability depression at  $F = 1$  or  $F = .5$ . The inbreeding is accomplished by making certain chromosomes completely homozygous, using a series of crosses using genetic markers and complicated inversions. In the latter method, on the other hand, the genetic load can be indirectly calculated by regressing the negative natural logarithm of viability on the inbreeding coefficient. This technique is used with considerably lower inbreeding levels. Applying these two independent techniques, many attempts have been made to evaluate the magnitude of genetic loads in various species of *Drosophila* and other organisms. It has been shown that the values estimated by these different techniques do not coincide for a given population. That is, the total homozygous load, measured by the GREENBERG and CROW method, is larger than the one calculated by the MORTON *et al.* method (DOBZHANSKY, SPASSKY and TIDWELL 1963; MALOGOLOWKIN-COHEN *et al.* 1964). This discrepancy could be partly explained by the existence of synergistic interaction at higher degrees of inbreeding. Considerable evidence for synergism among deleterious alleles at different loci in *Drosophila* has been accumulated recently (SPASSKY, DOBZHANSKY and ANDERSON 1965; KITAGAWA 1967; TEMIN *et al.* 1969; MUKAI 1969; KOSUDA 1971). In addition, the difference in the fitness measurements of these two methods should be considered. That is, the proportion of eggs reaching adulthood was used as the index in the MORTON *et al.* method, whereas the viability of a certain genotype, relative to that of other genotypes was employed in the GREENBERG and CROW

method. The MORTON *et al.* method was initially developed to estimate genetic loads in human populations, for which the degree of inbreeding is fairly low. Many authors have since used this method to estimate genetic loads, using inbreeding depression at comparatively low levels of inbreeding, i. e., from  $F = 0$  to .125 or .250.

The main purpose of the present study is to examine the effects of inbreeding on egg to adult viability over a wide range of inbreeding levels, and to present evidence for synergistic interaction among loci.

#### MATERIALS AND METHODS

Individuals of *Drosophila virilis*, a chromosomally monomorphic species, were collected from a natural population in a beer factory in Tokyo, Japan, in the autumn of 1970. Female flies were individually separated immediately after collection, and allowed to deposit eggs in separate culture vials. About eighty percent of the captured females had been inseminated in nature, and produced offspring. Thereafter, the isofemale lines were maintained by full sib-pair matings every generation. Parent flies were taken at random as far as possible. In order to keep these inbred lines without any loss from environmental causes, replicate crosses were made, and each pair of parents was transferred to a fresh vial every two days until progeny appeared. When one of the parents died, another pair mating was made so as not to lose the line. The eggs from the first sib-mating have an expected inbreeding coefficient of  $F = .250$ , if females were mated with single males in nature. And the progenies from the next three successive pair matings should have  $F$  equal to .375, .500, and .564, respectively. Offspring from the 6th sib-mating have  $F$  of .734. On the other hand, outbred progenies with an  $F$  value of 0 were produced from intercrosses between unrelated males and females taken from different lines at the first generation.

For measuring the egg to adult viability of inbred progenies from a given line, the following procedures were employed. Males and females from each inbred line were kept together for about one week after eclosion, so that they might achieve reproductive maturity. Then they were placed in a large plastic vial. A glass slide, with culture medium on its surface, was inserted into the vial. After several hours in the dark, a part of the medium with 50 eggs was separated, and was transferred to a standard culture vial with corn-meal-molasses medium. Four such 50-egg samples, a total of 200 eggs, were taken from each line for a given level of inbreeding. Complete counts of adult flies emerging from culture vials were performed. The breeding scheme is shown in Figure 1. The temperature was maintained at  $25 \pm 1^\circ\text{C}$  throughout the experiment.

#### RESULTS

The average viabilities, expressed as the proportions of eggs yielding adults, at the seven levels of inbreeding are presented in Table 1 and graphically shown in Figure 2. As shown in Table 1, the viability test for  $F = .672$  at the fifth pair mating was not conducted. The experiment was started with 117 inbred lines. Some of lines were lost, however, in the course of the experiment. After six generations of successive pair matings, the number of lines retained was reduced to 79. The number of lines lost is also given in Table 1. The viabilities of inbred flies were therefore calculated in two different manners. The first set of estimates (Analysis I) was computed, assuming that fitness of the flies in the lost lines were zero, although this assumption is unlikely to be justified. That is, it was taken for granted that the reasons for loss of inbred lines were exclusively genetic. The other set of estimates (Analysis II) was calculated excluding these lost lines. It was assumed that the loss of inbred lines was entirely due to non-genetic

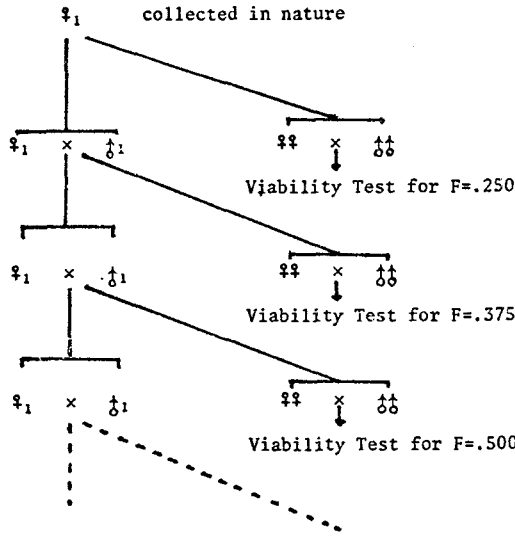


FIGURE 1.—Mating scheme.

causes. This assumption is also unlikely to be justified, since it neglects the existence of deleterious genes and sterility genes in this species. In fact, several inbred lines were lost because of distinctly incomplete development of testis. Accordingly, it is only natural that the viability computed by the first method is smaller than that calculated by the second.

As clearly seen in Table 1, the viability of outbred progeny is extremely high (94.1%), compared with other reports, indicating that culture conditions were nearly optimal. Table 1 shows that survival rate is a decreasing function of the

TABLE 1  
*Egg to adult viability in outbred and inbred progenies*

<i>F</i>	Viability	Number of lines	Number of lines lost	$-\log_e$ (Viability)
0	.9410 ± .0098	59		.0608
.250	.7774 ± .0112	117		.2519
.375	.6885 ± .0135	117		.3733
.500	.6313 ± .0163	117		.4600
	.6479 ± .0137*	114	3	.4341*
.594	.5379 ± .0222	117		.6202
	.5994 ± .0161*	105	9	.5119*
.734	.3632 ± .0277	117		1.0130
	.5379 ± .0221*	79	26	.6202*

\* These viabilities are calculated through Analysis II, excluding lost inbred lines, whereas other figures are obtained through Analysis I, including lost lines. For further explanation, see the text.

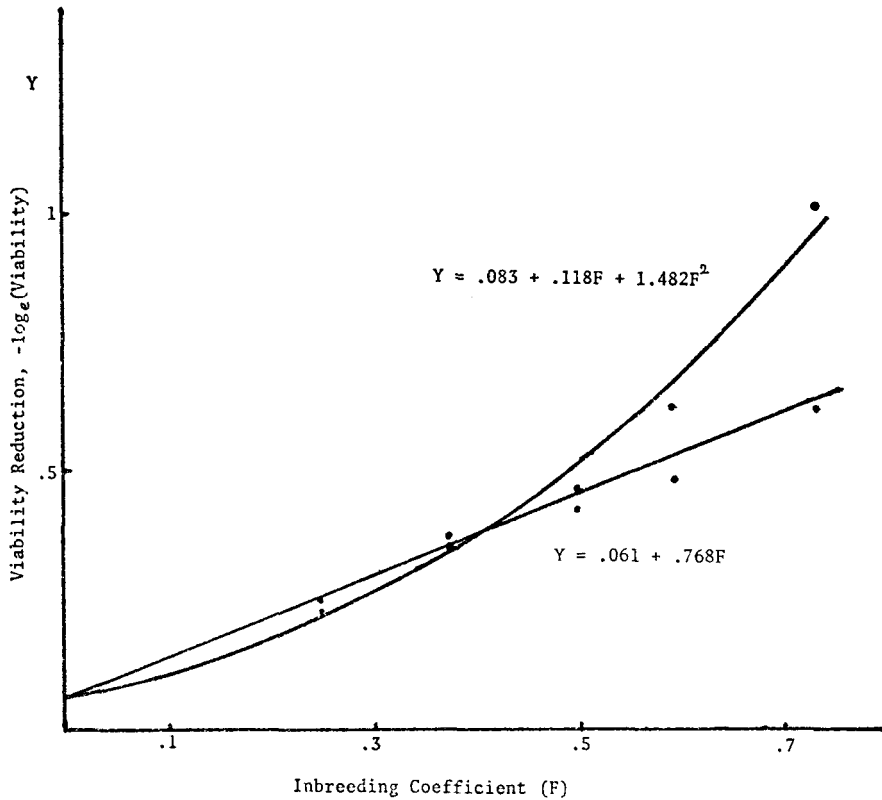


FIGURE 2.—Relationship between inbreeding and viability reduction,  $-\log_e$  (Viability).

level of inbreeding, much as expected. The variation in viability among lines increases as a function of  $F$ , although the mean viability decreases markedly with an increase in the value of  $F$ . This divergence with  $F$  should be mainly due to the fixation of different alleles and loci in different lines; and to differential viability effects at these loci. Actually, an analysis of variance has shown that the viabilities are highly heterogeneous among lines for a given level of inbreeding.

Figure 2 shows a synergistic interaction at high levels of  $F$  ( $F = .500$  to  $.734$ ), whereas the relationship between inbreeding and viability depression is almost linear below  $F = .500$ .

The magnitude of genetic load was evaluated from the data given in Table 1, with the use of weighted regression analysis (MALOGOLOWKIN-COHEN *et al.* 1964). Table 2 gives this estimated load, expressed in terms of  $A$  and  $B$ , together with  $B/A$  ratio, suggested by MORTON *et al.* (1956). The estimates of these quantities are presented in the bottom row of each section of Table 2, except for  $F = .734$ , for which losses were heavy. It is very important to note that the outbred load is remarkably and consistently small in comparison with many other studies in *Drosophila* hitherto reported (DOBZHANSKY *et al.* 1963; STONE, WILSON and GERSTENBERG 1963; MALOGOLOWKIN-COHEN *et al.* 1964; METTLER, MOYER and

TABLE 2  
A and B values and B/A ratios

	A	B	B/A
Analysis I	.058 ± .063	.865 ± .336	14.99
	.059 ± .063*	.828 ± .348*	14.10*
Analysis II	.061 ± .062	.768 ± .264	12.51
	.061 ± .062*	.770 ± .296*	12.56*

\* The viability data at  $F = .734$  are excluded from the calculation.

KOJIMA 1966). This may be expected, to some extent, because of the conspicuously high viability of outbred progenies. On the other hand, the amounts of inbred load are similar to those reported earlier in the literature. If the lethals (lost lines) are included, the inbred load increases, but if the estimates for  $F = .734$  are excluded, the load decreases. As a consequence, the  $B/A$  ratio becomes fairly large, because of the strikingly smaller value of  $A$ .

#### DISCUSSION

The mutational and segregational loads imposed on natural populations of diploid organisms result from recurrent mutation and from heterozygote superiority, respectively. The relative importance of these two loads in natural populations has been particularly controversial. MORTON *et al.* (1956) have shown, on theoretical grounds, that the  $B/A$  ratio has the value of unity when load is exclusively segregational, whereas it becomes much larger when load is entirely mutational and when the degree of dominance ( $h$ ) is small (cf. MUKAI and YAMAZAKI 1968). The  $B/A$  ratio has since been used by many authors as a criterion for determining whether the genetic load in populations is mainly segregational or mutational. This method, however, has been subjected to some criticism, especially with regard to two of its assumptions (CROW 1958; DOBZHANSKY *et al.* 1963; LEVENE 1963; MALOGOLOWKIN-COHEN *et al.* 1964; METTLER *et al.* 1966).

In the theory of the MORTON, CROW and MULLER method, it is presumed that the non-genetic death is negligible, compared with the genetic death, i.e., the  $B/A$  ratio is based on the assumption that total environmental death is zero. In reality, of course, the value of  $A$  includes the non-genetic death; if  $A$  includes a large portion of environmental death, then  $(A + B)/A$  no longer gives the proper ratio of inbred and outbred loads, and its value might be somewhat underestimated. It should be remembered here that the viability of outbred progenies is remarkably higher in the present study than in other studies;  $A$  consequently has a smaller value and the  $B/A$  ratio has a much larger value than in previous studies. These results clearly demonstrate that the estimate of  $B/A$  largely depends on the value

TABLE 3

*Estimates of A, B, and B/A, calculated for pairs of viability values of outbred and inbred progenies*

<i>F</i> values	Analysis I			Analysis II		
	<i>A</i>	<i>B</i>	<i>B/A</i>	<i>A</i>	<i>B</i>	<i>B/A</i>
0 and .250	.061	.765	12.58	.061	.677	11.13
0 and .375	.061	.833	13.71	.061	.803	13.21
0 and .500	.061	.798	13.13	.061	.720	11.84
0 and .594	.061	.942	15.52	.061	.711	11.69
0 and .734	.061	1.297	21.34	.061	.763	12.55

of *A*, since the *B* values are rather stable as mentioned earlier (METTLER *et al.* 1966). The extremely high survival rate at *F* = 0 in the present experiment suggests the possibility that considerable amounts of non-genetic death might be contained in the *A* estimates of other *Drosophila* studies, where survival rate at *F* = 0 is somewhat lower. If this conjecture is valid, then the resulting overestimates of *A* would serve as a very real limitation on the method of MORTON *et al.* (1956), wherever environmental conditions are not well controlled.

The MORTON, CROW and MULLER method also assumes that the contributions of different loci to overall fitness are independent and multiplicative. This, in turn, yields the linear regression equation of  $-\log_e(\text{Viability}) = A + BF$ . If the separate contributions of the various loci are not independent, however,  $-\log_e(\text{Viability})$  is no longer a linear function of *F*. Under these conditions, *B* is underestimated, and the *B/A* ratio may no longer be used as a criterion to distinguish between mutational and segregational loads.

Using only pairs of viability values of outbred and inbred progenies computed from Analysis I, the values of *A*, *B*, and the *B/A* ratio were estimated by the method of MORTON *et al.* (1956); these values are listed in Table 3. The increases of *B/A*, as a function of the value of *F* at which the viability of inbred lines is measured, indicates synergism among loci at the higher *F*'s. In the present study, the viabilities of inbred progenies have been estimated by two different methods, making two different assumptions about lost lines. Neither of these assumptions is likely to be justified. Some lines were probably lost because of genetic causes, while others were probably lost because of environmental causes; it is impossible to partition the total loss into two components, and consequently the proper regression line of viability reduction on *F* lies between the two lines:

- (1)  $-\log_e(\text{Viability}) = .061 + .768F$ , and
- (2)  $-\log_e(\text{Viability}) = .083 + .118F + 1.482F^2$ .

The deviations from linearity of equation (2) were significant (Table 4). The estimated viabilities at *F* = 1 were found to be .437 and .186 under models (1) and (2), respectively. The curvilinear regression line gives only the upper limit of viability reduction, as a function of *F*. It should be pointed out, however, that the effect of selection can not be avoided in the process of continued inbreeding. In the presence of selection, the *F* values estimated in this study should be over-

TABLE 4

*Analysis of variance for regression of  $-\log_e$  (Viability) on F*

Source	df	S. S.	M. S.	F
Regression	2			
Linear	1	.48855	.48855	122.44**
Quadratic	1	.04106	.04106	10.28*
Residual	3	.01198	.00399	
Total	5	.54159		

estimates, leading to underestimates of  $-\log_e$  (Viability) for high levels of inbreeding. The synergism would thus be underestimated. The degree of synergistic interaction can not be precisely determined in the present experiment. The curvature in the viability response, and hence the synergism, is not evident below  $F = .500$ , owing to the very small value of  $F^2$ . The synergism beyond  $F = .500$  was also suggested by CROW (1968).

Synergistic interaction in homozygous condition may be generally accepted, whereas experimental data on the interaction in heterozygous condition are scarce (KITAGAWA 1967; MUKAI 1969). In fact, synergism in heterozygous condition, especially for mildly detrimental genes, is more important in natural populations, because multiple locus homozygotes for deleterious alleles are rare in populations, and because the dominance of these detrimentals is large.

Taken at face value, the large  $B/A$  ratio in the present study argues for the greater relative importance of mutational load in this chromosomally monomorphic species than in other *Drosophila*. It is interesting to note that smaller  $B/A$  values were reported for *Drosophila pseudoobscura*, which is chromosomally highly polymorphic (DOBZHANSKY *et al.* 1963; STONE *et al.* 1963; METTLER *et al.* 1966). Furthermore, the experimental data for the "balance hypothesis" of population structure derives mainly from this species (DOBZHANSKY and SPASSKY 1963; GREENBERG and CROW 1960). It might be suspected that the nature of genetic load is related to the extent of the structural heterozygosity.

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