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# HOMOZYGOUS AND HEMIZYGOUS VIABILITY VARIATION ON THE *X* CHROMOSOME OF *DROSOPHILA MELANOGASTER*

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Manuscript received May 21, 1985
Revised copy accepted August 2, 1985

ABSTRACT

We report here a study of viability inbreeding depression associated with the *X* chromosome of *Drosophila melanogaster*. Fifty wild chromosomes from Mt. Sinai, New York, and 90 wild chromosomes from Death Valley, California, were extracted using the marked *FM6* balancer chromosome and viabilities measured for homozygous and heterozygous females, and for hemizygous males, relative to *FM6* males as a standard genotype. No statistically significant female genetic load was observed for either chromosome set, although a 95% confidence limit estimated the total load <0.046 for the samples pooled. About 10% of the Death Valley chromosomes appear to be "supervital" as homozygotes. There is little evidence for a pervasive sex-limited detrimental load on the *X* chromosome; the evidence indicates nearly identical viability effects in males and homozygous females excluding the supervital chromosomes. The average degree of dominance for viability polygenes is estimated between 0.23 to 0.36, which is consistent with autosomal variation and implies near additivity. We conclude that there is little genetic load associated with viability variation on the *X* chromosome and that the substantial reduction in total fitness observed for chromosome homozygosity in an earlier study may be due largely to sex-limited fertility in females.

STUDIES of whole chromosome, fitness-related inbreeding depression in Drosophila have played a significant role in the formulation and testing of the classical and balance views of genetic variation in natural populations (SIMMONS and CROW 1977; LEWONTIN *et al.* 1981). However, rejecting even extreme versions of these single-locus selection models is confounded by the fact that multiple locus (whole chromosome) and not single locus variation is measured. Observed inbreeding depression at that level is compatible with either model (LEWONTIN 1974). Consequently, the ubiquitous depression of partial or *total* fitness measures associated with the inbreeding of the major autosomes can be attributed to either a mutational load under purifying selection or a segregational load generated by overdominant selection, or both to varying degrees.

In sex-linked or haplo-diploid genetic systems, purifying selection should rapidly eliminate recessive deleterious mutations in the haploid or hemizygous state, theoretically resulting in a markedly reduced mutational load in natural

populations. Hemizygosity forces partial dominance of otherwise recessive mutations with respect to selection. Conversely, while it has been shown theoretically that heterosis or overdominance *per se* is not sufficient for the maintenance of an *X*-linked polymorphism (BENNETT 1958; HALDANE and JAYAKAR 1964; CANNINGS 1967; AVERY 1984), the constraints imposed by the fitnesses in males do not necessarily mean that overdominant loci will be greatly reduced on the *X* chromosome (CURTSINGER 1980). Therefore, the presence of a significant load associated with the *X* chromosome would favor overdominance.

With this argument in mind, WILTON and SVED (1979) studied the effects of *X* chromosome homozygosity on *total* fitness, using a design similar to the cage approach used to study total fitness depression for autosomes (SVED and AYALA 1970; TRACEY and AYALA 1974). From these relative comparisons they estimated an average 40% reduction in fitness associated with *X* chromosome homozygosity. Assuming additivity for single-locus effects, and doubling this value because the *X* chromosome is half the size of either autosome, this estimate is remarkably similar to the 80–90% reduction in total fitness observed in the autosomal studies. Given this observation, it must be argued that there are a large number of *X*-linked loci with overdominant fitnesses in females or that the detrimental load is largely sex-limited. The autosomal studies had, in fact, ascribed a large fertility contribution to the total load, as the egg-to-adult viability component measured in most studies was considerably less. It is expected that fertility contributions will be mostly sex-limited as sterility appears to be caused by separate sets of loci in each sex (LINDSLEY and GRELL 1968). Furthermore, sex-limited loci will contribute a disproportionately greater share to the mutational load on a per-locus basis (CROZIER 1976). As there is no reason to presume that a viability contribution will be sex-limited, it seems reasonable that viability variation may contribute little to the total fitness reduction observed for the *X* chromosome.

While egg-to-adult viability depression has been studied extensively for the autosomes (SIMMONS and CROW 1977), few studies have characterized inbreeding depression for viability on the *X* chromosome in Drosophila. Virtually no study exists in which such a load is characterized in any detail, in spite of the fact that such a study could shed light on the issues of mutational and segregational loads. The intrinsic absence of lethals on the *X* chromosome (DUBININ 1946) and the complications imposed by detrimentals that may be sex-limited in their effects has undoubtedly contributed to the lack of *X* chromosome viability studies. While the absence of *X*-associated lethals in natural populations is an empirical observation, the possibility of a significant sex-limited viability component is largely speculative. It is logical that hemizygosity in Drosophila males will rapidy eliminate detrimental mutations with similar expression in both sexes, while detrimental mutations with effects confined to females (sex-limited) will persist at levels similar to the autosomes. However, it does not necessarily follow that a significant portion of the genome has the physiological potential to be sex-limited with respect to viability.

The existence of a significant X-linked and sex-limited genetic load with respect to viability has been claimed in earlier studies. The investigations of

KERR and KERR (1952), DRESCHER (1964) and GALLO (1978) all set out to examine sex-limited viability variation on the *X* chromosome of *Drosophila melanogaster*, and each reported significant levels (8–16%) of "sex-limited" inbreeding depression in homozygous females. However, as elaborated in the DISCUSSION section, all three studies have problems in their experimental design. Given these reservations, we feel the problem of sex-limited viability variation associated with the *X* chromosome must still be considered an open issue.

We report here a study of *X*-chromosome-associated viability variation in two temperate natural populations of *D. melanogaster*. We show that there is little sex-limited or nonsex-limited viability reduction. In addition, we quantify the genotypic variance for variability, its average degree of dominance and the correlation of genotypic viability variation between the sexes.

## MATERIALS AND METHODS

*Extraction*: Wild male *D. melanogaster* flies were collected late in October 1981 from Davis Peach Farm, Mt. Sinai (MS), New York, and in March 1982 from Furnace Creek Ranch, Death Valley (DV), California. The Death Valley collection was kindly provided by J. A. COYNE and S. ORZACK. Chromosomes were first extracted from wild males by crossing with *C(1)DX, y f* compound-*X* females, and the $F_1$ males were electrophoresed for the *G6PD* and *6PGD* loci. A subsample of chromosomes was then extracted by crossing $F_1$ males with several $FM6/N^{264-84}$ females and discarding the $N^{264-84}$ females in the second generation ($N^{264-84}$ is homozygous and hemizygous lethal). In this fashion, 50 and 90 chromosomes were randomly extracted from the MS and DV collections, respectively. The *FM6* chromosome is a multiply inverted balancer chromosome possessing *B* (bar eyes), *y* (yellow body), *dm* (diminutive) and *sc* (scute) mutations (LINDSLEY and GRELL 1968). *FM6* homozygous females are viable, but sterile.

*Viability estimation*: The viability of individual *X* chromosomes in homozygous and heterozygous combination was determined by subjecting each chromosome to a standard genetic scheme and counting the emerging offspring by genotype. In each inbred cross, five virgin $X_i/FM6$ females were mass mated with five $X_i$ males to constitute a single replicate cross.

Each cross was replicated twice in the MS study and four times in the DV study. In each study replicate crosses were placed in eight-dram vials with food for 4 days and were transferred to a second vial for another 4 days before the adults were discarded. Outbred crosses were set up in an identical fashion, except that different $X_i$ and $X_j$ chromosomes were combined at random to constitute the same number of random cross combinations as used in homozygous line estimates. Consequently, two independent estimates of male hemizygous viability were available for each chromosome; one from the inbred and one from the outbred female viability crosses. Crosses for both inbred and outbred estimates were constructed simultaneously.

The viabilities of males and females were determined separately relative to *FM6* males as a standard genotype. In emerging $F_1$ progeny, the ratio of the four genotypes (*FM6* and $X_i$ males, and $FM6/X_i$ and $X_i/X_i$ females) under Mendelian segregation and equal sex ratio, is expected to be 1:1:1:1. We expressed the relative viability of $X_i/Y$ and $X_i/X_i$ genotypes as the ratio of the number of wild-type males or females to the number of *FM6* males plus one (HALDANE 1956). Since *FM6* males have clearly reduced viability due to mutant markers, unstandardized viability estimates average about 1.5 to 2.0.

Flies emerging from each vial were counted on days 12, 15 and 18, and the counts for each pair of sequentially established vials from each replicate cross were pooled and are used as single viability observations.

Before analysis, all raw viability estimates were divided by the mean of the outbred crosses so that viability measured for all genotypes was relative to the average random heterozygote. This sets the mean of the outbred crosses at 1.0. Replicate crosses were used as the within cross error variance, and analysis of variance (ANOVA) was carried out on the untransformed viability data

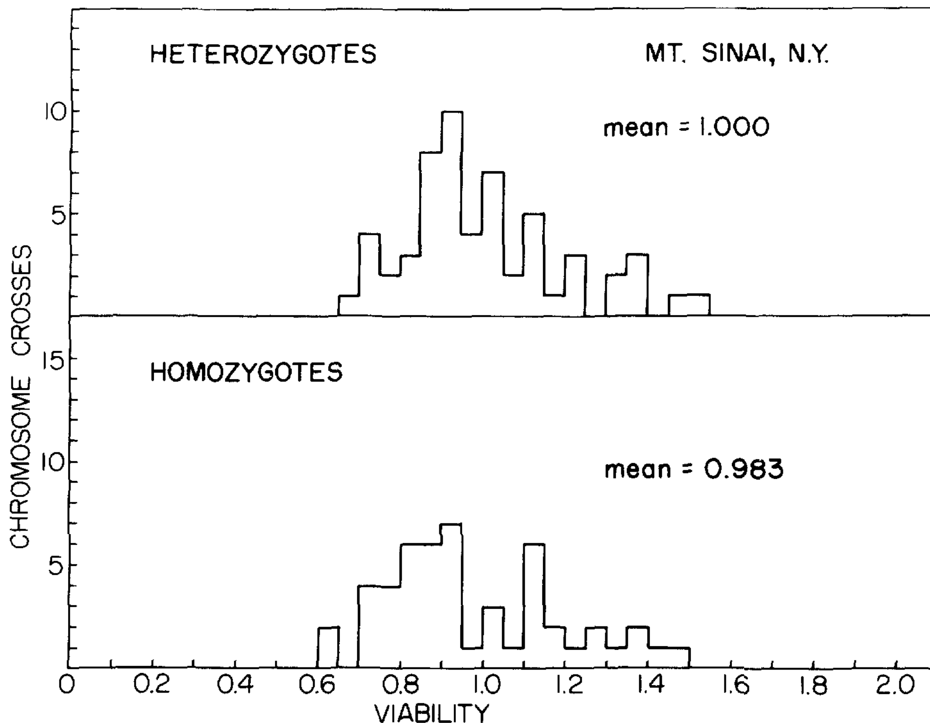


FIGURE 1.—The distribution of homozygote and heterozygote relative viabilities for 50 *X* chromosomes from Mt. Sinai, New York. Viabilities are standardized so that the mean heterozygote viability is 1.000.

in tests for significant genotypic variance. Estimates of the genotypic variance component came directly from the ANOVA. The empirical viability distributions and associated tests do not strictly conform to the assumptions of ANOVA. Nevertheless, we do not consider this a problem, except in cases where tests give marginal statistical significance. Tests of inbreeding depression were carried out using a *t*-test for unequal sample sizes and unequal variances (SOKAL and ROHLF 1969).

All flies and crosses were reared in eight-dram shell vials and were given standard cornmeal food, as described in MUKAI (1964), except that Karo corn syrup was substituted for molasses. Experiments were carried out at 25°.

## RESULTS

*Distribution of homozygote and heterozygote viabilities and the genetic load*: In total, both experiments involved the counting of over 167,000 offspring for 273 different crosses using the 140 wild *X* chromosomes. Figures 1 and 2 illustrate for both Mt. Sinai (MS) and Death Valley (DV) the distributions of *X* chromosome homozygote and heterozygote viability estimates relative to *FM6* males and divided by their respective heterozygote means. In both collections, mean female homozygote viabilities were not statistically different from heterozygote viabilities. Mean homozygote viabilities were 0.981 ± 0.030 and 1.013 ± 0.032 in the MS and DV samples, respectively.

No lethal or semilethal chromosomes were recovered; however, the procedure of starting with wild-caught males precluded recovering this general class

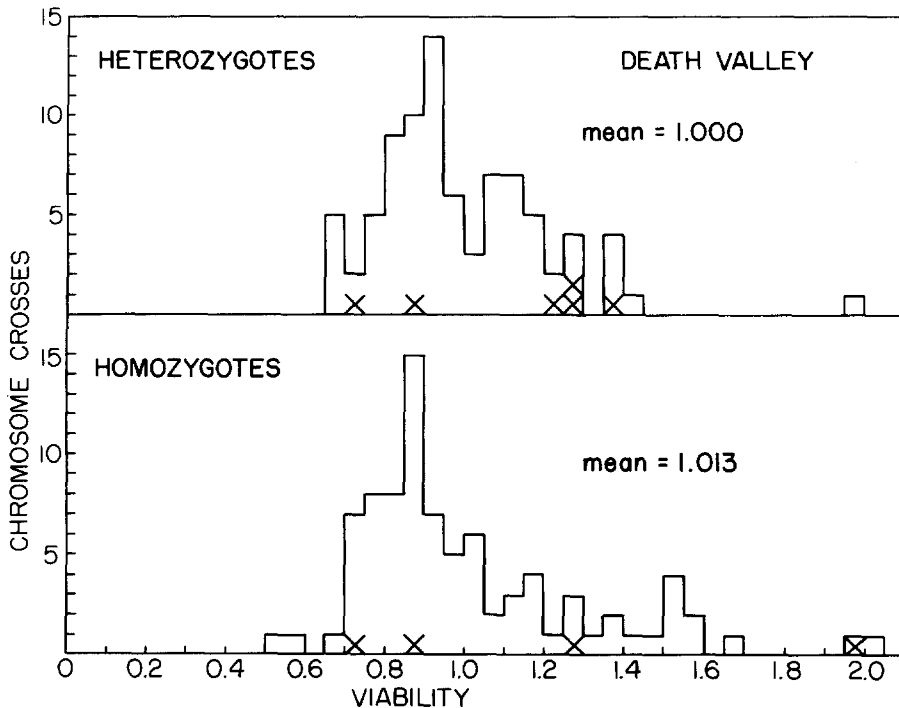


FIGURE 2.—The distribution of homozygote and heterozygote relative viabilities for 90 $X$ chromosomes from Death Valley, California. X's mark the outliers as identified in the text.

of chromosomes. Female-limited lethal chromosomes would have been recovered under this scheme, but none were seen. An independent collection of wild females was made the following year from MS in October 1982. Each female, although nonvirgin, was mated with *FM6* males, and 148 independent wild chromosomes were ultimately extracted with the *FM6* balancer. Three lethal-bearing chromosomes were recovered for a lethal chromosome frequency of 0.020. GALLO (1978) estimated lethal frequency from a collection of 203 $X$ chromosomes at 0.025. Combining these two estimates, we will assume the typical lethal level to be about 2.2%.

In the MS sample, no statistically significant genotype variance in viability, $V_G$, was detected in homozygous females, heterozygous females or hemizygous males, although the genotypic variance for homozygous crosses was near significance ($V_G = 0.0078$, $F_{49,47} = 1.56$, $P < 0.10$). In contrast, the DV collection showed statistically significant genotypic variance in homozygous crosses ($V_G = 0.068$, $F_{86,248} = 4.2814$, $P < 0.0001$), heterozygous crosses ($V_G = 0.031$, $F_{87,258} = 3.026$, $P < 0.002$) and hemizygous males (pooled estimates from inbred and outbred crosses, $V_G = 0.044$, $F_{173,506} = 3.659$, $P < 0.001$). The genotypic variance of female viability in homozygous crosses was twice that observed for heterozygous crosses ($F_{86,87} = 2.19$, $P < 0.005$). Examination of the actual viability distributions for DV (Figure 2) shows a skew toward high viability or "supervital" chromosomes, especially in the homozygous crosses, where two

chromosomes had homozygous viabilities twice the average heterozygote viability.

The possibility that these particular chromosomes constitute spurious experimental "outliers" was considered by generating a bivariate plot of male chromosome viabilities as measured independently in inbred and outbred sets of crosses. The correlation between these two measures was 0.526 ($P < 0.001$), and four chromosomes could be identified as having significantly different male viabilities in effectively replicate crosses. Ten crosses were involved where the two hemizygous estimates were statistically different ($P < 0.05$). Approximately this proportion is expected by chance. It is apparent from Figure 2 that the supervitals are generally not outliers as identified in this fashion.

The inbreeding depression (as a difference in means) for both chromosome sets is not statistically different from zero. For MS with a smaller number of chromosomes and replicates, the mean difference is −0.019, with a lower 95% confidence limit of −0.084. The inbreeding depression for the DV chromosomes was +0.013, with an associated lower estimate of −0.0396. Exclusion of the outlier crosses from this sample actually increases the mean homozygote viability. Conversely, dropping of the supervital tail (viabilities ≥ 1.30) sharply decreases the genotypic variance, as expected, ($V_G = 0.016$) and lowers the estimate of inbreeding depression to −0.053 (not significant) and its associated 95% limit to −0.106. Since this exclusion is arbitrary, we shall include this supervital set in the analyses that follow, bearing in mind that they are possibly obscuring a small inbreeding depression.

The inbreeding depression for both chromosome samples pooled is not statistically significant from zero (mean difference = 0.003 ± 0.02) and has a combined lower 95% confidence limit estimate of −0.0496, which also cannot be statistically excluded. In conclusion, we emphasize that the inbreeding estimates are not statistically different from zero, but will refer to the estimated level by its statistical limits (≤0.0496) in the discussions that follow.

It is useful to frame the viability inbreeding depression associated with the *X* chromosome in the context of genetic load (MORTON, CROW and MULLER 1956; GREENBERG and CROW 1960), and to contrast it with genetic loads observed for the autosomes. The total genetic load, $T$, for data from both sets of *X* chromosomes combined is computed as $T = \ln(A/C) = \ln A - \ln C$, where $A$ and $C$ are the viabilities of heterozygotes and homozygotes, respectively (GREENBERG and CROW 1960). Using the lower 95% limit of the inbreeding depression, the total load is $T \leq 0.072$ with lethal studies and is ≤0.046 without lethals. In autosomal studies the total load is generally divided into lethal ($L$) and detrimental ($D$) contributions which are additive. The detrimental load $D$ is often further partitioned into semilethal ($D_s$) and mild detrimental loads ($D_m$). The latter is usually attributed to the effects of viability polygenes (MUKAI and YAMAGUCHI 1974) and, by definition, includes all chromosomes with homozygous viabilities greater than 0.60. A summary of genetic load studies by SIMMONS and CROW (1977) provides the following average estimates for autosomal studies; $T = 0.484$, $D = 0.236$, $L = 0.247$, $D_m = 0.134$. It would appear that the total genetic load on the *X* chromosome, once adjusted for

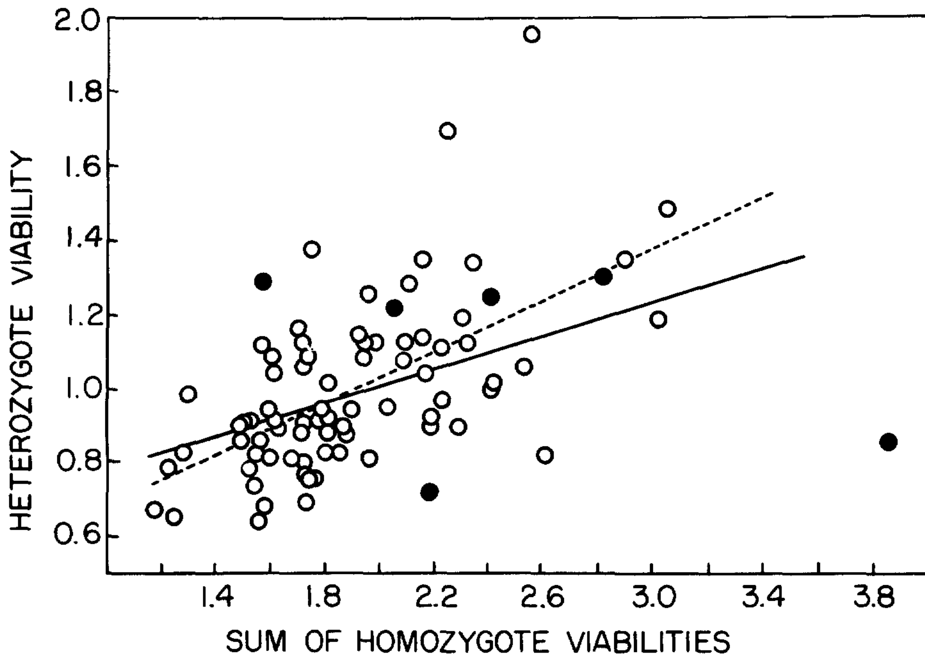


FIGURE 3.—The relationship between heterozygote viability and the sum of the corresponding homozygote viabilities in the crosses using the chromosomes from Death Valley. Filled circles represent crosses identified as outliers, as discussed in the text. The solid and dashed lines represent the associated regression with and without outliers, respectively.

size, is about one-third of the total load (includes lethal estimates) and, at the most, two-thirds of the mild detrimental load (excluding lethal estimates) observed for the autosomes. Again, it should be emphasized that this is, at best, an upper estimate to the load, as statistically there is no inbreeding depression. This is, of course, consistent with the model of purifying selection on the *X* chromosome, where any load will be rapidly eliminated in males.

*Estimate of the average degree of dominance for viability*: The average degree of dominance of mutant viability polygenes for the DV chromosomes was estimated, assuming a Wrightian fitness model, by the regression of individual heterozygote viabilities against the sum of the respective homozygote viabilities constituting each cross (see MUKAI *et al.* 1972; MUKAI and YAMAGUCHI 1974). The slope of the regression line approximates the average degree of dominance, $h$, of mutant polygenes in an equilibrium population. A plot of the viabilities and the associated regression lines, both including and excluding the outliers identified above, is shown in Figure 3. The estimated degree of dominance is $0.230 \pm 0.050$ and $0.356 \pm 0.050$ with and without outliers, respectively. This suggests that most of the polygenic viability mutations carried in these chromosomes are nearly additive in their expression. No analysis of the MS chromosomes was carried out because of the absence of any significant genotypic variance in viability.

*Correlation between hemizygous and homozygous viabilities*: There is a clear cor-

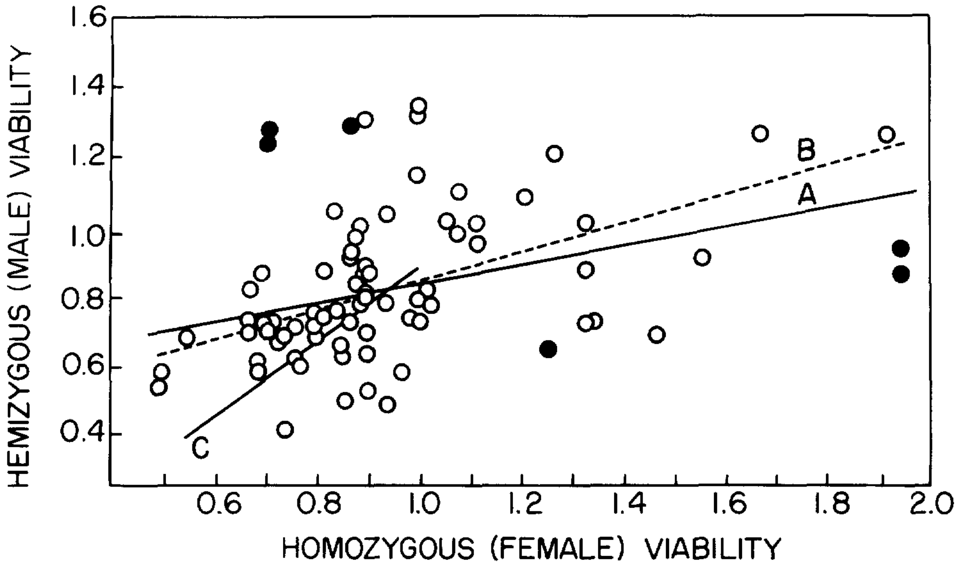


FIGURE 4.—The relationship between homozygote female viabilities and the corresponding hemizygous viability for the chromosomes from Death Valley. Hemizygous viabilities come from the same chromosomes used in the outbred crosses. Regression lines A and B represent least square fits with and without outliers. Line C represents the regression for all homozygous viabilities <1.000. (See text for values.) Filled circles represent crosses identified as outliers, as discussed in the text.

relation ($r = 0.36$, $P < 0.001$) between male hemizygous and female homozygous viability indices for the DV chromosomes. We have used male viabilities from outbred crosses, rather than inbred crosses, to assure complete statistical independence of the two estimates. Sampling both viabilities from the inbred crosses would have violated this assumption, as both viability estimates include the same *FM6* males. The MS collection was not analyzed because of the lack of statistically significant hemizygous or homozygous genotypic variance. The slope of the regression between these indices, as shown in Figure 4, is 0.265 ± 0.075 and 0.410 ± 0.080 with and without outliers, respectively, and reflects the substantially larger genotypic variance in viabilities among homozygote (females) crosses ($F_{86,173} = 1.64$; $P < 0.005$). This, by itself, might suggest some sex-limited component to viability, with mutations possessing smaller effects in males on the average. However, consideration of the bivariate distribution modifies this conclusion.

The increased variance in females arises from the "supervital" homozygous chromosomes, which have little or no correlation with their respective male viabilities. If we arbitrarily examine the relationship between homozygous and hemizygous viabilities in those female chromosomes with homozygous viabilities less than 1.00 (mean heterozygous viability), we see effectively equal expression in both sexes (Figure 4, line C, slope = 0.727 ± 0.182). In fact, it would appear that there is equal effect for all chromosomes with homozygous viabilities <1.30.

## DISCUSSION

Earlier studies (KERR and KERR 1952; DRESCHER 1964; GALLO 1978) claimed to demonstrate sex-limited inbreeding depression associated with the *X* chromosomes of *D. melanogaster.* In those studies, the viability of females in homozygous or heterozygous combination was measured as the ratio of females to males in the same crosses (number of females per 100 males). The study of KERR and KERR (1952) observed a ratio of 98.7 females to 100 males for homozygous female progeny, and DRESCHER (1964) estimated 95.6 females per 100 males. Neither study carried out suitable numbers of crosses using randomly combined X chromosomes as an estimate of heterozygous ratios. The KERR and KERR (1952) study included only five crosses using heterozygous combinations of chromosomes (104.8 females per 100 males). They carried out no statistical tests, but using the error estimate obtained in their inbred experiment, this value is not statistically different from their inbred mean. DRESCHER (1964) carried out no random crosses, but referenced both the KERR study and the study by l'HERITIER and TEISSIER (1936), which reported the "normal" sex ratio to be 106.8 females per 100 males. The absence of suitable numbers of random heterozygote crosses internal and parallel to each study makes conclusions about sex-limited load equivocal in these investigations. Control or outbred ratios reported in this study, and from the studies of GALLO (1978) and l'HERITIER and TEISSIER (1936), range from 120.84 to 100.98 females per 100 males. Clearly, there is no standard sex ratio that may be used as a control value for these experiments.

The GALLO (1978) study included a large number of random X chromosome crosses for which the sex ratios were compared with the inbred crosses. The sex ratio was 92.1 females/100 males in the inbred group and was 110.0 females/100 males in the outbred group. These differences are statistically significant using the error variance estimates available from our studies. However, the random crosses were not established using the same design. The inbred crosses were segregating for the *M-5* balancer chromosome, whereas the outbred progeny crosses were free of this balancer. Because of this, the inbred crosses also possessed 50% higher densities. Both of these considerations lead us to question the suitability of the inbred-outbred experimental comparison in that study.

Our studies contrast the relative viabilities of both homozygous and random heterozygous combinations of X chromosomes and use the *FM6* male genotype internal to each cross as a standard or reference genotype, allowing the estimation of other genetic parameters, such as the degree of dominance and the correlation between homozygous and hemizygous viabilities. We find no statistically significant sex-limited or nonsex-limited inbreeding depression for viability associated with homozygosity for the *X* chromosome. If we accept the statistical limits of our combined samples and assume these results are typical for the *X* chromosome, then we cannot reject a general inbreeding depression of about 5% or less. We may ask if this level is compatible with levels of inbreeding associated with the major autosomes, assuming the classical model.

In principle, the haplo-diploid system imposes partial dominance on all mutants such that the effective degree of dominance under selection is equal to $(2/3)h + 1/3$. Therefore, we expect the respective deleterious loads for both types of chromosomes to assume similar levels as the dominance of a mutant class increases. Empirical data suggests that the average degree of dominance of new mutants is inversely related to the average selective effect in homozygotes (SIMMONS and CROW 1977). Therefore, we might expect convergence in the component loads, the milder the class of mutants contributing to it. Consequently, the high degree of dominance of viability polygenes predicts that the mild detrimental loads for both genetic systems should be similar, although still lower for the *X* chromosome. The ratio of the two loads should reflect the ratio of effective dominances.

The deleterious viability load $D_m$ exposed upon inbreeding can be defined as the product of the average viability effect ($s$) and the equilibrium frequency of the mutant ($q$), summed across all contributing loci on the chromosome, $D_m = \sum q_i s_i$. For an autosomal locus $qs = \mu/h$, where $h$ is the average degree of dominance and $\mu$ is the mutation rate. For a sex-linked locus, $h$ is replaced by $(2/3)h + 1/3$. Since we assume $\mu$, $h$ and $s$ for newly arisen mutants to be similar regardless of the genetic system, the ratio of sex-linked to autosomal loads is $h/[(2/3h + 1/3]$. Therefore, for the class of mutants with $h = 0.20$, we predict a ratio of $(0.20)/(0.40) = 0.50$, or a sex-linked load of about one-half the autosomal load contributed by mutants of this class. The best available estimates of the average degree of dominance of mild detrimentals (viability polygenes) in equilibrium populations are 0.178–0.480 (MUKAI and NAGANO 1983), and the average estimate of the mild detrimental load in autosomal studies is 0.134 (SIMMONS and CROW 1977). Taking into account the fact that the *X* chromosome is half the size of either autosome, we predict $D_m$ loads on the *X* chromosome of 0.026–0.049. These values are consistent with our observation of a load, $D \leq 0.046$.

The possibility remains that some of the observed load on the *X* chromosome is segregational, arising from a limited number of overdominant loci. There is enormous latitude in the number of loci potentially contributing to the total load of the autosomes, but the small total viability load of the *X* chromosome sharply limits the number of heterotic loci for viability. Generally, for an overdominant locus, the inbred viability load as measured from the random-bred population mean (heterozygotes) is

$$L = [\tilde{s}\,\frac{(k-1)}{k}]\,n$$

where $k$ is the number of segregating alleles, $\tilde{s}$ is the harmonic mean of individual locus selection on homozygotes and $n$ is the number of segregating loci (MUKAI *et al.* 1974).

As the total viability load on the *X* chromosome is less than 0.046, this limits the number of overdominant loci (assuming $k = 2$) on the *X* chromosome to 112 with $\tilde{s} = 0.001$ or to 12 with $\tilde{s} = 0.01$. Consequently, single locus overdominance must be either very weak ($\tilde{s} < 0.001$) or the number of overdom-

inant loci associated with viability must be a very small fraction of the estimated 1000 to as many as 4000 genes on the *X* chromosome (SPIERER *et al.* 1983; JUDD, SHEN and KAUFMAN 1972, BOSSY, HALL and SPIERER 1984).

In theory, X-linked systems are limited in their ability to maintain polymorphism, as overdominant selection can only operate in females. Moreover, fitness configurations in which hemizygous selection in males is similar in direction but more intense than in homozygous females further reduce the ability to protect polymorphism, even with overdominance in females. However, such specific fitness configurations also constrain polymorphism at autosomal loci. Unless the *X* chromosome has a greater propensity to acquire these fitness configurations, it should have fewer overdominant loci only because the opportunity for overdominance in males is not available. There is little empirical evidence to suggest that selection should be more intense in males. The very fact that the X chromosome has evolved dosage compensation argues that selection has operated to equalize effects in both sexes (HARTL 1971). Assuming selection is similar in the homozygous and hemizygous genotypes, then the treatment by CURTSINGER (1980) predicts the opportunity for polymorphism in haplo-diploid systems to be about one-half that of diploid systems if nonsex-limited and equal for sex-limited loci in females. We cannot reject the argument that the small load associated with the *X* chromosome has a significant overdominant component. Nevertheless, the limits to the number of heterotic loci outlined in the preceding paragraph still hold.

The possibility exists that some of the genotypic variance is contributed by elevated mutation arising from hybrid dysgenesis generated in our crosses (ENGELS 1983). The original *C(1)DX* attached-*X* stock is *P* cytotype due to repeated outcrossing to wild lines; however, the $FM6/N^{264-84}$ stock is *M* cytotype. The direction of the cross with that strain is potentially dysgenic; therefore, we cannot reject the possibility that *de novo* mutations are contributing to the measured load. It should be pointed out that, out of the 140 chromosomes extracted using this scheme, none acquired a new lethal, when several might have been expected, given observed rates in a known dysgenic system. This suggests that our crosses were not as dysgenic as expected. Nevertheless, the possible presence of additional mutation reinforces our contention of very little genetic load for viability on the *X* chromosome in natural populations.

In this study we assume Mendelian segregation or the absence of significant segregation distortion, either derived naturally or through dysgenic events (MATTHEWS and HIRAIZUMI 1978; HIRAIZUMI 1979). Such distortion will affect the genotypic variance, the correlation between sexes and the estimate of the degree of dominance, but will not, in principle, generate inbreeding depression; it will distort the genotypic proportions in both inbred and outbred crosses.

This experiment was not designed to uncouple and quantify the effects of segregation distortion. This would have involved a significant increase in complexity (see CURTSINGER 1984). Most studies involving autosomes have also suffered from this problem. However, with respect to this study certain facts are pertinent. First, with segregation distortion of the type associated with

dysgenic events, male recombination is involved and the distortion is associated with immediate dysgenic germline events (HIRAIZUMI 1979). When tested, our chromosomes were two generations past the putative dysgenic cross; therefore, segregation distortion cannot be attributed to immediate dysgenic germline events. Second, we may examine the possibility of a strong pervasive segregation distortion associated exclusively with one sex (*i.e.*, Sex Ratio) by partitioning the regression of heterozygote against the sum of homozygote viabilities (Figure 3) into maternally and paternally derived contributions. Segregation distortion could contribute to such a correlation because distortion expressed in the parental germlines will perturb genotype (or sex) ratios in the same direction in both homozygous and heterozygous crosses involving that chromosome. For example, an *X* chromosome meiotically driven in males will distort in the same direction apparent viability estimates in both homozygous and heterozygous female offspring, leading to the correlation seen in Figure 3. If we reexamine this regression, not using the sum of the homozygote viabilities but, rather, examining the regression of heterozygote values on the homozygote values of paternally and maternally contributing chromosomes separately, we may be able to detect a large segregation distortion associated with one sex exclusively. The regression coefficient of heterozygote viability on homozygous viability when the chromosomes were transmitted paternally was $0.242 \pm 0.080$, while the coefficient when the chromosomes were transmitted maternally was $0.284 \pm 0.079$. The similar regressions argue against the presence of a strong segregation distortion associated with these *X* chromosomes and acting exclusively in one sex.

In conclusion, if we assume our sets of naturally reared X chromosomes to be comparable with those used by WILTON and SVED (1979), it would appear that both sex-limited viability depression and overdominance play a minor role in the total reduction in fitness. Our estimate put inbreeding depression at zero, possibly as large as 5%, leaving female fertility as the most plausible major contributor to the inbreeding depression observed by those authors. The study by TRACEY and AYALA (1974) concluded that fertility effects in both sexes contributed substantially more than viability to the total reduction in fitness that they observed. Since much of this component must be sex-limited, it is not unreasonable that the large reduction in fitness associated with homozygosity of the *X* chromosome can be attributed to a substantial female fertility component.

We would like to thank LESLIE MCDONOUGH, VESNA PRODAN and MARY SERAPHIN for their assistance in several aspects of this study, as well as the owners of Davis Peach Farm for access to their orchards. JERRY COYNE and STEVE ORZACK kindly provided flies from Death Valley. GWEN LUKE was very patient in typing the manuscript. This study was supported, in part, by National Science Foundation Grant DEB 8106930 to W. F. EANES. Contribution 305 from the Program in Ecology and Evolution, State University of New York, Stony Brook.

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Communicating editor: M. T. CLEGG