

The inbreeding decline and average dominance of genes affecting male life-history characters in *Drosophila melanogaster*

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(Received 2 March, 1994 and in revised form 12 September, 1994)

Summary

This paper describes the results of assays of male life-history characters in a large outbred laboratory population of *D. melanogaster*. Lines of flies homozygous for the entire third chromosome and lines of flies carrying two different third chromosomes were assayed for age-specific male mating ability (MMA), age-specific survivorship, male fertility, and body mass. The results of these assays were used to calculate the inbreeding decline associated with each of these traits, the average dominance of deleterious alleles that affect the traits, the genotypic and environmental components of variance for the homozygous lines, and phenotypic and genotypic correlations among the characters. Significant inbreeding decline was found for all characters except the Gompertz intercept and fertility. Early and late MMA show larger effects of inbreeding than any other trait. The inbreeding load for MMA is about the same magnitude as that for egg-to-adult viability, but is substantially less than that associated with total fitness. The estimated inbreeding decline and average dominance of male life-history characters are comparable to estimates for other *Drosophila* fitness components.

1. Introduction

The deleterious consequences of inbreeding for normally outbreeding organisms have long been recognized (Darwin, 1868; Darwin, 1876; Wright, 1977). Experiments on the effects of inbreeding in *Drosophila melanogaster* represent the best available data on the causes and consequences of inbreeding in animals. Even so, only a limited number of characters have been investigated. Most studies have involved assays of egg-to-adult viability, or population-cage estimates of net fitness. Few investigations have been conducted on the effects of inbreeding on suites of life-history characters (Simmons & Crow, 1977).

Several investigators have attempted to decompose the within-population variability for total fitness into components associated with measurable life-history characters (Prout, 1971; Bundgaard & Christiansen, 1972; Anderson *et al.* 1979; Wilkinson, 1987). These studies have suggested that variability for male characters, especially male reproductive success, contributes more to variability for total fitness than do traits such as female fecundity or egg-to-adult viability.

One male life-history trait, mating success, exhibits inbreeding decline at least as severe as that associated with egg-to-adult viability (Pendlebury & Kidwell, 1974; Brittnacher, 1981; Kosuda, 1983; Sharp, 1984; Partridge *et al.* 1985). These results also suggest that male reproductive success may be more genetically variable than other fitness components. The inbreeding decline associated with other male life-history characters has not been extensively studied.

Estimates of the average dominance coefficient of detrimental alleles in natural populations have also been chiefly derived from studies of egg-to-adult viability in *Drosophila* (Simmons & Crow, 1977; Crow & Simmons, 1983). Results from these studies indicate that alleles with mildly deleterious effects on viability have average dominance coefficients characteristic of partially recessive alleles. We would like to know if the estimates provided by studies of viability are generalizable to other components of fitness, and to fitness itself.

To address these issues, this paper presents the results of assays of male life-history characters in a large outbred laboratory population of *D. melanogaster*. Lines of flies homozygous for the entire third chromosome and lines of flies carrying two different third chromosomes were assayed for age-specific male

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mating ability, age-specific survivorship, male fertility, and body mass. The results of these assays were used to calculate (1) the inbreeding decline associated with each of these traits, (2) the average dominance of deleterious alleles that affect the traits, (3) the genotypic and environmental components of variance for the homozygous lines, and (4) phenotypic and genotypic correlations among the characters as expressed in chromosomal homozygotes.

2. Methods

(i) Creation of parental lines

The base population (*IV*) was derived from a collection of about 200 fertilized females made by the late Dr P. T. Ives in Amherst in 1975, that was subsequently maintained as a large population. Twenty-one (inversion-free) isofemale lines from the *IV* stock were used to found the current base population in 1977. Since then, it has been maintained as a randomly mating laboratory stock of large population size (several hundred individuals). Repeated quantitative-genetic assays of sterno-pleural bristle number in this population suggest there has been little erosion of genetic variability over time (B. Charlesworth, unpublished data). Details of the maintenance of these lines can be found in Charlesworth & Charlesworth (1985).

In order to create a suitable third-chromosome balancer stock that would not introduce extraneous variability into the experimental lines, the third-chromosome balanced lethal system *TM6/Sb* was placed on an *IV* genetic background, producing a stock which is $+/+$, $+/+$, *TM6/Sb* for the X, second, and third chromosomes, respectively (+ represents a wild-type chromosome derived from the *IV* population). The *TM6* chromosome contains multiple, overlapping inversions that function as an effective suppresser of crossing-over for the third chromosome (Lindsley & Zimm, 1992). The *TM6* chromosome also contains the dominant marker, *Dichaete* (*D*), allowing the identification of individuals bearing the chromosome, and visible recessive mutations that allow detection of crossover events. The resulting balanced-lethal system was then used to produce lines of flies carrying isogenic third chromosomes. Different lines were intercrossed in a North Carolina II breeding design (Comstock & Robinson, 1952) to generate the experimental material.

Each extraction involved crosses to at least nine different females from the balancer stock. Each extraction line was then subdivided and maintained as two independent sublines. The sublines were expanded and maintained in 5–10 shell vials (20–50 flies per vial) for 2–3 generations before the matings were performed. In any given experimental cross, flies from only one subline were used; the other subline was used in the replicate cross for that genotype. Consequently,

common environmental effects and effects of common genetic background within a subline will contribute to the variance within lines, and not to the variance among lines. This experimental protocol avoids confounding effects of third chromosomes with effects attributable to other chromosomes. These are included in the 'environmental' variance, which may therefore be somewhat inflated. The effect is probably small, however, since many females from the balancer stock were used as parents in the first two generations of the extraction procedure (the variance due to such effects of the genetic background is $1/n$ times the corresponding genetic variance, where n is the number of maternally-derived diploid genotypes per line).

I thus produced many independent lines of flies. Each line segregated for the balancer and an independently-extracted third chromosome. The wild-type chromosomes can be maintained intact through many generations, due to the lack of crossing over in balancer heterozygotes. All flies used as parents of experimental males possessed *IV* X and second chromosomes derived from the balancer stock. The *IV* stock contains the *I* and *P* elements responsible for hybrid dysgenesis, as shown by Dr D. K. Hoffmaster (pers. comm.) using standard genetic methods (Engels, 1989). By the rules of inheritance of cytotype, the balancer stock with X chromosomes from the *IV* stock is of the I/P cytotype. That is, crosses between females of this strain and males derived from the *IV* population should not induce a high mutation frequency in the offspring.

(ii) Genetic analysis of heterozygous and homozygous genotypes

Forty chromosomal lines of genotype (*TM6/+*) were divided into five groups of eight lines. Each group was assayed as a block, and was set up at a different time over the course of 18 months. Each block of eight lines was subdivided into two subgroups of four lines each. Females from lines in subgroup 1 were then crossed to males of lines in subgroup 2 and *vice versa*. Blocks thus consisted of 16 different heterozygous genotypes, and genotypes were represented by two replicates from each of two reciprocal parental crosses (a total of four replicates). For each line in a block, some females were crossed to males from their own line, to produce genotypes homozygous for the entire third chromosome. Two independent replicate homozygous crosses were made for each line. Homozygous individuals produced in this manner have an inbreeding coefficient, $F = 1$ for the entire third chromosome, whereas heterozygotes have $F = 0$ for the third chromosome. Assays of all replicates of the homozygous lines and corresponding heterozygotes within a block were conducted simultaneously. Figure 1 shows a diagram of the crossing scheme.

All wild-type progeny of every cross had identical genotypes with respect to the third chromosome.

		Block 1								...	Block 5																														
		Dams																																							
Sires	Chromosome lines	1	2	3	4	5	6	7	8	...	33	34	35	36	37	38	39	40	...	33	34	35	36	37	38	39	40														
		1	o				x	x	x	x																															
	2		o			x	x	x	x																																
	3			o		x	x	x	x																																
	4				o	x	x	x	x																																
	5	x	x	x	x	o																																			
	6	x	x	x	x		o																																		
	7	x	x	x	x			o																																	
	8	x	x	x	x				o																																
	...																																								
	33										o					x	x	x	x																						
	34											o				x	x	x	x																						
	35												o			x	x	x	x																						
	36													o		x	x	x	x																						
	37										x	x	x	x	o																										
	38										x	x	x	x		o																									
	39										x	x	x	x			o																								
	40										x	x	x	x				o																							

Fig. 1. North Carolina II Breeding design. Series of crosses for a modified NCII breeding program. Each 'x' represents a cross between females of the line represented by the column number and males of the line represented by the row numbers; 'o' is used to represent crosses that result in flies homozygous for chromosome III. Every cross is of the form $TM6/+_j \times TM6/+_k$. The wild type males produced from each cross will have third-chromosome genotype $+_j/+_k$. These genotypes are constructed by crossing males from lines represented by column numbers to females from lines represented by row numbers. Each 'o' represents a cross between females and males from the same line. The wild-type males produced from these crosses will have third-chromosome genotype $+_j/+_j$.

Within each replicate, male flies from several independent cultures were assayed to control for bias due to common environment. To control for density effects, larvae were cultured at low density. (No more than three females laid eggs in any vial, and all females were removed after two days of oviposition.) Flies were collected from all replicate crosses for assays of age-specific mating ability, age-specific survivorship, mean longevity, fertility, and body mass.

For the heterozygous lines, this breeding scheme allowed me to partition the variability for each trait into components associated with the additive effects of genes (V_A), non-additive effects, environmental effects (V_E), and effects associated with reciprocal crosses (Hughes, ms. in press). Since no significant reciprocal effects were found for any of the traits, reciprocal crosses were treated as simple replicates. For homozygous crosses, the variance could be partitioned into components associated with genotypic (V_G) and environmental effects. The estimates of V_A and V_G were used to calculate the average dominance coefficient associated with genes affecting each trait (see Section 2v). The inbreeding decline associated with each trait was calculated by comparing trait values in homozygous lines to their values in heterozygous lines.

(iii) Character assays

Details of the protocols for character assays are given in Hughes (ms. in press). All replicates of each heterozygous and homozygous genotype were assayed for weekly survivorship and mean longevity by placing 20 males of a given genotype together in a vial. Dead experimental flies were replaced with flies carrying a visible mutation, to maintain a constant density. Flies were transferred to fresh vials weekly, at which time survivors were counted and dead flies were replaced with males from the mutant stock. The slope and intercept of the linear regression of log mortality on age gives the so-called Gompertz parameters. This model is based on the empirically observed tendency for mortality rates in many animals to increase exponentially with age, such that

$$p(t) = \exp\left\{\frac{A(1 - \exp(Bt))}{B}\right\}, \tag{1}$$

where $p(t)$ is the probability of survival from time zero to time t , A is the Gompertz intercept and B is the Gompertz slope (L. Mueller, pers. comm.). The Gompertz intercept is a measure of a baseline or 'intrinsic' mortality rate of a genotype. The Gompertz slope estimates the instantaneous slope of the curve relating log (mortality) to age, and has been used as a measure of the accelerating effect of senescence on the risk of dying in a given time interval (Finch, 1990; Hughes & Charlesworth, 1994).

Mating trials were conducted by allowing 10 virgin males of each replicate experimental genotype to compete with 10 virgin *scarlet* (st/st) males for matings with 10 virgin st/st females. Male mating ability (MMA) was then scored as the proportion of the 10 st females that produced wild-type offspring. MMA was assayed for 3-day old males and for 21-day old males.

Relative male fecundity or 'fertility' was assayed by mating females from the st stock either to males from the st stock or to males from one of the replicate vials for a given experimental genotype. One female of each type was then placed into a fresh vial, where the two females were allowed to oviposit for 3 days. Fertility was scored as the proportion of wild-type progeny, out of the total progeny number. Although this estimate of fertility confounds actual fecundity with differences in egg and larval viability, it nevertheless provides an estimate of reproductive success that is independent of mating success.

Live body mass was measured for equal-aged groups of male flies that had been maintained at density of 10 flies per vial since eclosion. When it was not possible to weigh 10 flies for each replicate, all available flies were weighed. The mean body mass of flies in the group was recorded.

All characters were scored as described above and were standardized relative to the character mean for the block when computing genetic and genotypic variances and covariances and phenotypic correla-

tions. This standardization was performed in order to minimize inter-block effects. No lines were included in an analysis for a given character if its mean for that character was less than 50% of the heterozygous mean. Such lines may carry severely deleterious mutations that have different properties from mildly deleterious mutations (Crow & Simmons, 1983).

(iv) *Variance component estimation*

Standard ANOVA techniques were used to compute genetic and environmental variance components for the male life-history traits. Genotypic variance components for homozygous lines were estimated as the variance component among lines (Sokal & Rohlf, 1981, p. 197), pooled over all five blocks:

$$V_G = \frac{1}{n_0} (MS_{\text{between}} - MS_{\text{within}}) \quad (2)$$

in which n_0 is the number of replicate observations for each homozygous line, MS_{between} is the mean square among lines, and MS_{within} is the mean square within lines. The value of n_0 is adjusted for unequal sample sizes by the formula given by Sokal & Rohlf (1981, p. 214). Environmental components of variance were estimated as the residual mean squares (MS_{within}). Because mating trials and fertility trials were binomial processes, the expected binomial variance of the trials could be calculated from the mean mating success (or fertility) and the harmonic mean sample size of each type of trial.

To compute genetic and environmental components of variance from the assays of heterozygous lines, a factorial ANOVA, modified for unequal cell numbers, was performed for all characters, and variance components due to general and specific combining abilities of different chromosomes were calculated by the synthesis method (Hughes, ms. in press). Causal components of genetic variance are calculated directly from the variance components of general and specific combining abilities that are obtained from this analysis (Kempthorne, 1957, p. 451).

Genetic and environmental coefficients of variation (CV_G and CV_E) for the homozygous lines were calculated. The coefficient of variation of a distribution is the standard deviation divided by the mean and multiplied by 100. It provides a measure of the magnitude of variability relative to the trait mean. It is a dimensionless metric, and is the most appropriate statistic for the comparison of variation that was originally measured on different scales or in different characters (Houle, 1992). Because all traits were standardized to a mean value of 1, CVs are calculated as the square root of the variance component $\times 100$.

Genotypic correlations between characters were computed from the variance components by the formula:

$$\text{Cov}(x, y) = \frac{1}{2}(\sigma_{x+y}^2 - \sigma_x^2 - \sigma_y^2), \quad (3)$$

where x and y are the two traits and σ_i is the genotypic standard deviation of trait i (Kempthorne, p. 265). The genotypic correlation, r_G , is then given by

$$r_G = \frac{\text{Cov}(x, y)}{\sigma_x \sigma_y}. \quad (4)$$

The r_G contain contributions from additive and from non-additive genetic effects. Phenotypic correlations (r_P) among traits in the homozygous lines were estimated as the product-moment correlation coefficients, calculated from all observations in all blocks of the analysis. Coefficients of covariation were calculated as exact analogs of coefficients of variation: as the square root of the covariance (or its absolute value, if negative, with the sign restored after taking the square root) standardized by the trait mean and multiplied by 100. They express the degree of covariance between two traits relative to the trait means.

(v) *Calculation of inbreeding decline and average dominance coefficient*

Inbreeding decline or male life-history traits will be expressed as $(1 - [w_i/w_o])$, where w_i is the mean value of a given fitness component measured in the heterozygous lines, and w_o is the value measured in homozygous lines. Line means were used in the calculations to avoid bias due to unequal sample sizes among lines. Because the Gompertz intercept and slope estimate mortality and the rate of increase of mortality with age, respectively, their values increase with decreasing fitness. Transformations of these variables were therefore necessary so that the ratio of homozygote to heterozygote values would be less than one, if homozygotes were less fit than heterozygotes. The Gompertz intercept was transformed to $A_{\text{max}} - A_i$, where A is given by equation (1), A_{max} is the maximum value of A over all lines in the analysis, and A_i is the value of A for individual heterozygous or homozygous lines. The Gompertz slope was similarly transformed to $B_{\text{max}} - B_i$, where B is given in equation (1), and the subscripts have the same meaning as those for the Gompertz intercept. The values for A_{max} and B_{max} for this set of lines were 6.041×10^{-3} and 0.150, respectively.

A method of estimating the average dominance coefficient for rare alleles with deleterious effects on a fitness component is given by Mukai *et al.* (1972). Their method involves measurement of the character in chromosomal homozygotes and in the heterozygotes produced by crosses between different homozygotes. Any two homozygotes and their corresponding heterozygote contain no more than two alleles at any given locus. In the one-locus, two-allele case, let 'A' represent the 'normal' allele and let the deleterious allele be represented by 'a'. The frequency of 'a' is assumed to be $\ll 1$, consistent with its being

maintained by mutation-selection balance. The relative mating ability (or other fitness component) of individuals of different genotypes can be expressed as:

Genotype:	AA	Aa	aa
Relative 'fitness'	1	1-hs	1-s.

The coefficient, *h*, indicates the degree of dominance of the deleterious allele.

Mukai *et al.* (1972) showed that, for multiple loci, the regression of the heterozygous fitness on the sum of the fitnesses of the two homozygotes is equal to a weighted average of *h* over all loci (\bar{h}). The weights are proportional to the genetic variance of the homozygotes at each locus that contributes to variation in the character. This result is dependent upon the assumption that $h > 0$.

This method was used here to estimate of \bar{h} for male life-history traits. Because the measurements of homozygous and heterozygous traits were carried out in five different blocks, independent estimates of \bar{h} were calculated for each block. A mean value was then calculated over the five independent estimates. Following Mukai *et al.* (1972), severely deleterious homozygous lines were excluded from the analysis (see above).

Because the regression method typically yields estimates with large sampling errors, a second method of calculation was also employed. Assuming additivity across loci and that $h > 0$, we can express genetic components of variance among heterozygous and homozygous lines as

$$V_A = \sum 2q(hs)^2 = \sum 2u(hs) \tag{5}$$

and

$$V_G = \sum qs^2 = \sum \left(\frac{u}{h}\right)s, \tag{6}$$

where *q* is the frequency of allele 'a', *u* is the haploid mutation rate, $q = u/hs$ under mutation-selection balance with $h > 0$, V_A is the additive genetic variance of the heterozygous lines, and the summation is over all loci. Assuming that there is no correlation between *u*, *h*, and *s*, it is easily demonstrated that

$$\frac{V_A}{V_G} = 2\bar{h}^2 \tag{7}$$

in which \bar{h} represents the average of *h* over all loci. If *s* and *h* are negatively correlated, as suggested by the *Drosophila* viability data (Crow & Simmons, 1983), then V_A/V_G is given by

$$\frac{V_A}{V_G} = 2\bar{h}^2 \left(1 + \frac{2C}{\bar{h}\bar{s}}\right), \tag{8}$$

where *C* is the covariance between *h* and *s*. This method will therefore give an underestimate of \bar{h} if the covariance between *h* and *s* is large relative to the

product of \bar{h} and \bar{s} . Equation (7) has been used to derive estimates of \bar{h} for the male life-history traits.

(vi) *Statistical analyses*

Estimates of inbreeding decline were calculated from the ratio of homozygous to heterozygous performance. The ratios were calculated over all heterozygous lines for all blocks. The mean estimate for each block was determined either by (1) calculating this ratio for the estimate for each heterozygous line and the mean estimate for the two corresponding homozygous lines, then averaging these ratios over the lines for which the necessary data was available, or (2) calculating the mean score for all heterozygous and all homozygous lines in a block, averaging over blocks, then calculating the ratio. For most traits, approximately 40 heterozygous lines could be matched with measurements of both homozygotes. Only these lines were used for the first method. The second calculation was made in order to test for possible bias in the first method caused by the exclusion of many heterozygous lines for which one or both homozygotes were missing. The heterozygous lines within each block are not independent of one another, since many share a third chromosome derived from one of the parental lines. In order to construct valid statistical tests, a *t*-test was conducted on the mean difference between fitness component estimates for heterozygotes and homozygotes from the five independent blocks in the experiment.

Significance of the \bar{h} estimates derived from the regression method (hereafter designated as \bar{h}_1) and the variance-component method of Equation 7 (\bar{h}_2) was determined by treating estimates from each block as independent estimates of the population value. Since *t*-tests are relatively insensitive to deviations from normality (Remington & Schork, 1970), they were used to determine if the \bar{h}_1 estimates were statistically distinguishable from zero. This test was conducted by comparing the mean estimate over the five blocks with the standard error derived from the empirical variance of the estimates between blocks.

Significance of the V_G estimates for homozygous lines was determined by conducting a standard *F*-ratio test on the results of a separate nested ANOVA for each character. Blocks and lines-within-blocks were used as the independent variables, and the score for the character was treated as the dependent variable in these analyses. Assumptions of ANOVA methods were verified by inspection of residuals, and, if necessary, transformations were applied to the dependent variables for significance testing. Therefore, significance tests for standardized early and late MMA were based on the analysis of log-transformed data. Other significance tests were based on the analysis of untransformed variables. All the mean squares and variance components were calculated from the untransformed data.

Table 1. Estimates of inbreeding decline in male fitness components*

Trait	Heterozygous lines ^a	Heterozygous lines ^b	Homozygous lines	Inbreeding decline		Standard	
				Method a	Method b	Error ^a	Paired <i>t</i> -test ^a
Body Mass	73.817	72.936	71.428	0.024	0.030	0.0066 (<i>n</i> = 65)	3.057 (<i>P</i> = 0.019)
Early MMA	0.516	0.532	0.427	0.171	0.198	0.0377 (<i>n</i> = 38)	5.707 (<i>P</i> = 0.002)
Late MMA	0.456	0.457	0.276	0.312	0.396	0.0450 (<i>n</i> = 35)	4.281 (<i>P</i> = 0.006)
Slope of Mortality	0.091	0.093	0.109	0.169 ^c	0.184 ^c	0.0534 (<i>n</i> = 42)	3.699 (<i>P</i> < 0.001)
Intercept of Mortality	1.51 × 10 ⁻³	1.72 × 10 ⁻³	1.87 × 10 ⁻³	0.021 ^d	0.030 ^d	0.0726 (<i>n</i> = 42)	1.107 (<i>P</i> = 0.137)
Mean Longevity	78.437	77.736	71.926	0.081	0.075	0.0114 (<i>n</i> = 42)	5.679 (<i>P</i> = 0.005)
Fertility	0.631	0.628	0.636	-0.007	0.006	0.0850 (<i>n</i> = 29)	0.041 (<i>P</i> = 0.515)

* Means of heterozygous and homozygous lines and standard errors are calculated over all heterozygous lines in all blocks. Inbreeding decline is calculated by the two methods described in the text (Section 2v). The Gompertz slope and intercept were transformed as described before calculation of the inbred ratio. Numbers in parentheses after the standard errors indicate the number of heterozygous lines that were included in the sample. *t*-tests and probability values are calculated from tests on the estimates from five independent blocks (four blocks for fertility estimates). *t*-values are calculated on the difference between heterozygote and homozygote means for each block. *P* values are those associated with a one-tailed test.

^a Heterozygous line mean is calculated from those heterozygous lines for which both corresponding homozygous lines were measured.

^b Averaged over all lines in all blocks.

^c Inbreeding decline calculated as $(B_{\max} - B_{\text{hom}})/(B_{\max} - B_{\text{het}})$ (see text).

^d Inbreeding decline calculated as $(A_{\max} - A_{\text{hom}})/(A_{\max} - A_{\text{het}})$ (see text).

Significance tests for genetic covariances were calculated by treating the estimates from each block as independent estimates of the population values. The significance of the r_G values was based on the probability values for the genetic covariances. No separate statistical tests for r_G were performed. Tests of significance for the estimates of r_p were calculated by computing Fisher's *z*-transformation (Sokal & Rohlf, 1981, p. 583). Probability values for r_p and for the genetic covariances were subjected to the sequential Bonferroni correction for multiple tests (Rice, 1989).

3. Results

(i) Inbreeding depression

Mean values for male life-history traits in heterozygous and homozygous lines, along with the corresponding estimates of inbreeding decline, appear in Table 1. Significant inbreeding decline was found for all characters except the Gompertz intercept and fertility. The two different estimates of inbreeding depression are similar for all characters, indicating that little bias resulted from the restriction of one estimate to a subset of the available heterozygous lines. The statistical tests reported in Table 1 are those for the estimates of inbreeding decline based on the heterozygous lines for which both corresponding homozygous lines were measured. Statistical tests on the alternative measure of inbreeding decline produced similar results.

The two estimates of MMA are associated with moderately severe inbreeding depression. Although body mass, mean longevity, and the Gompertz slope

also show significant inbreeding decline, the effects of homozygosity for chromosome III are less severe for these characters than for either early or late MMA. The baseline mortality rate (given by the Gompertz intercept) is higher in homozygotes than in heterozygotes, although the difference is not significant, and the magnitude of inbreeding decline for this character is very similar to that for mean longevity and the Gompertz slope. The only character for which there is no suggestion of inbreeding decline is fertility. This contrasts with the significant inbreeding decline reported for a similar fertility assay in Partridge *et al.* (1985).

(ii) Average dominance coefficients

Estimates of \bar{h} derived from both methods of calculation are shown in Table 2. \bar{h}_2 values for body mass, early MMA, and mean longevity are significantly different from 0 by two-tailed *t*-tests of the estimates from each block (an estimate for fertility could not be calculated by this method, because the VA estimate for this character was negative). The \bar{h}_1 estimates for the Gompertz slope and for mean longevity are also significantly different from zero. The two alternative methods yield generally similar values. The most disparate estimates are those for body mass. Even for this trait, however, the sampling errors of the estimates are large enough to account for the difference. Except for the regression estimate for fertility, point estimates of \bar{h} for all characters fall between 0 and 0.5. Values between 0 and 0.5 are those associated with partially recessive alleles. 95% confidence limits about the \bar{h}_1 estimates for body mass, early MMA, Gompertz slope and intercept, and

Table 2. Estimates of weighted average dominance for male life history traits, computed by two different methods*

Trait	Method of h estimation	\bar{h}	S.E.	t -test ($h = 0$)	Lower 95% c.l.	Upper 95% c.l.
Body Mass	1	0.080	0.144 ($n = 5$)	0.554 ($P = 0.609$)	-0.321	0.481
	2	0.420	0.108 ($n = 4$)	4.203 ($P = 0.012$)	-0.111	0.801
Early MMA	1	0.071	0.116 ($n = 5$)	0.616 ($P = 0.571$)	-0.250	0.392
	2	0.263	0.326 ($n = 5$)	2.104 ($P = 0.052$)	-0.219	1.593
Late MMA	1	0.266	0.159 ($n = 5$)	1.677 ($P = 0.169$)	-0.175	0.707
	2	0.500	0.410 ($n = 4$)	1.844 ($P = 0.103$)	-1.007	2.517
Slope of Mortality	1	0.138	0.038 ($n = 5$)	3.662 ($P = 0.022$)	-0.033	0.241
	2	0.349	0.169 ($n = 2$)	1.478 ($P = 0.189$)	-1.894	2.393
Intercept of Mortality	1	0.093	0.093 ($n = 5$)	0.995 ($P = 0.376$)	-0.166	0.351
	2	0.241	0.117 ($n = 2$)	1.063 ($P = 0.240$)	-1.365	1.614
Mean Longevity	1	0.301	0.116 ($n = 5$)	2.586 ($P = 0.061$)	-0.022	0.624
	2	0.397	0.182 ($n = 5$)	3.449 ($P = 0.013$)	0.122	1.133
Fertility	1	-0.155	0.141 ($n = 4$)	1.093 ($P = 0.354$)	-0.605	0.296
	2	—	—	—	—	—

* For each trait, the first row represents estimates of \bar{h}_1 and the second row represents estimates of \bar{h}_2 . Estimates were calculated for each block separately, and the mean value over blocks is reported. The standard errors, one-tailed t -tests, confidence limits (c.l.) and probability values are based on the independent estimates from each block. Numbers in parentheses after the standard errors indicate the number estimates available for each analysis. \bar{h}_2 for fertility was not estimable by the method of equation 7, because the estimate of V_A for this character was negative. The estimate of V_G for body weight, late MMA, and the Gompertz parameters were negative in at least one block of the analysis, so \bar{h} was estimable by the variance component method for fewer than five blocks for each of these characters.

Table 3. Variance components and coefficients of variation of all traits, derived from the homozygous lines*

Trait	MS _{between}	(D.F.)	MS _{within}	(D.F.)	P	V_G	CV_G	V_E	CV_E
Body Mass	0.0052	32	0.0022	25	0.017	0.0017	4.1	0.0022	4.7
Early MMA	0.1037	21	0.0305	23	0.001	0.0406	20.1	0.0305	17.5
Late MMA	0.0915	11	0.0437	16	0.248	0.0385	19.6	0.0915	20.9
Slope of Mortality	0.2243	25	0.1690	20	0.074	0.0333	18.2	0.1690	41.1
Intercept of Mortality	3.5062	25	2.1563	20	0.120	0.8132	90.2	2.1563	146.8
Mean Longevity	0.0437	24	0.0060	20	< 0.001	0.0227	15.1	0.0060	7.7
Fertility	0.0684	18	0.0501	16	0.264	0.0106	10.3	0.0501	22.4

* The expected binomial variances of early MMA trials, late MMA trials, and fertility trials were 0.1595, 0.3175, and 0.0113, respectively. P -values shown are those obtained before correction for multiple tests.

fertility do not overlap 0.5. All the \bar{h}_2 estimates have 95% confidence limits that include 0.5.

Male fertility is the only character for which the point estimate of \bar{h}_1 (-0.155) suggests that many segregating alleles may be overdominant in their effects. However, the confidence limits around this estimate are large, and the estimate cannot be bounded away from values representative of recessive or partially recessive alleles.

(iii) Genetic variances and covariances

Genetic components of variance for three of the seven characters (body mass, early MMA, and mean longevity) are significantly greater than zero, and that for the Gompertz slope is marginally non-significant (Table 3). Genetic variance components for late

MMA, the Gompertz intercept, and fertility were not significant. Late MMA and fertility had fewer lines represented in the analysis of variance components than did the other traits, because many homozygous lines caused semi-sterility or very low mating success and were excluded from the analysis. If corrections for multiple tests are applied, the values for early MMA and mean longevity remain significant at $P < 0.05$.

Of the 40 homozygous chromosome lines produced in this experiment, seven proved to be lethal or sublethal. A conservative definition of lethality was employed such that only homozygous lines that produced fewer than 10 wild-type males were classified as lethal or sublethal. The frequency of sublethals under this classification (18%) is comparable to other estimates of the frequency of lethal chromosomes in *Drosophila melanogaster* (Greenberg & Crow, 1960).

Table 4. Phenotypic correlations among characters in inbred lines*

Trait	Body mass	Early MMA	Late MMA	Slope of mortality	Intercept of mortality	Mean longevity
Early MMA	-0.113 (-0.397, 0.190)	—	—	—	—	—
Late MMA	-0.082 (-0.400, 0.253)	0.112 (-0.220, 0.421)	—	—	—	—
Slope of Mortality	-0.078 (-0.381, 0.238)	0.026 (-0.295, 0.343)	-0.202 (-0.515, 0.158)	—	—	—
Intercept of Mortality	-0.082 (-0.383, 0.236)	0.118 (-0.209, 0.421)	0.060 (-0.295, 0.400)	-0.613** (-0.764, -0.398)	—	—
Mean Longevity	0.112 (-0.207, 0.409)	0.180 (-0.148, 0.473)	0.012 (-0.338, 0.359)	-0.272 (-0.516, 0.013)	-0.261 (-0.508, 0.025)	—
Fertility	0.270 (-0.070, 0.553)	-0.294 (-0.579, 0.054)	0.022 (-0.347, 0.386)	-0.059 (-0.417, 0.314)	0.258 (-0.120, 0.571)	-0.294 (-0.597, 0.081)

* Tests of significance were calculated by computing Fisher's z-transformation. Probability values for r_p were subjected to the sequential Bonferroni correction for multiple tests. Numbers in parentheses are 95% confidence intervals.

** Significant at $P < 0.01$ by the sequential Bonferroni method applied to the entire table.

Table 5. Genotypic coefficients of covariation between characters measured in inbred lines*

Trait	Body mass	Early MMA	Late MMA	Slope of mortality	Intercept of mortality	Mean longevity
Early MMA	4.3 (4.04)	—	—	—	—	—
Late MMA	8.2 (4.81)	15.2† (6.06)	—	—	—	—
Slope of Mortality	-4.3 (4.67)	-15.1† (10.89)	12.7 (—)	—	—	—
Intercept of Mortality	-2.3 (10.48)	— (—)	— (—)	— (—)	—	—
Mean Longevity	2.8 (2.94)	7.2 (8.23)	6.7 (13.27)	-11.3 (8.59)	— (—)	—
Fertility	-10.9† (1.02)	-13.1 (—)	— (—)	— (—)	— (—)	-11.6 (10.00)

* Covariances that were inestimable because one of the variance component estimates was zero or negative are indicated by the symbol (—). Numbers in parentheses below CV estimates are the empirical standard errors from the five independent blocks.

† Significant at $P < 0.05$ before Bonferroni correction.

This result provides further evidence that the base population has not lost much genetic variability during laboratory culture.

Phenotypic correlations among the characters are reported in Table 4. Only one estimate of r_p remains significant after correction for multiple tests: the negative correlation between the Gompertz slope and the Gompertz intercept ($r_p = -0.613$). The two Gompertz parameters were estimated from the same data, and are not independent estimates of survivorship. The phenotypic correlation between these characters suggest that flies that have high baseline mortality rates have age-specific mortality rates that increase slowly with age. Relatively large, though non-significant, correlation coefficients in Table 4 suggest possible phenotypic relationships between

early MMA and fertility (negative), body mass and fertility (positive), late MMA and the Gompertz slope (negative), and between mean longevity and both Gompertz parameters (both negative).

Tables 5 and 6 report genetic components of covariation and estimates of r_G among traits, respectively. Of 18 genotypic coefficients of covariation that were tested for significance, three had probability values less than 0.05. Covariance between body mass and fertility, between early MMA and late MMA, and between early MMA and the Gompertz slope are significant by this criteria. When a sequential Bonferroni correction is applied to the probability values, all become non-significant. However, with 18 tests, fewer than one type-1 error is expected. If taken at face value, these covariances suggest: (1) genotypes

Table 6. Genotypic correlations (r_G) between characters expressed in the homozygous lines*

Trait	Body mass	Early MMA	Late MMA	Slope of mortality	Intercept of mortality	Mean longevity
Early MMA	0.618 (0.486)					
Late MMA	0.822 (0.596)	0.970† (1.015)				
Slope of Mortality	-0.217 (0.133)	-0.934† (0.102)	0.463 (—)			
Intercept of Mortality	-0.035 (0.348)	(—)	(—)	(—)		
Mean Longevity	0.174 (0.408)	0.317 (3.965)	0.157 (0.808)	-0.461 (0.358)	(—)	
Fertility	-2.368† (0.238)	-0.813 (—)	(—)	(—)	(—)	-0.708 (0.835)

* Correlations that were inestimable because one of the variance component estimates was zero or negative are indicated by the symbol (—). Numbers in parentheses below r_G estimates are the empirical standard errors from the five independent blocks. Significance of correlations is based on the significance of genotypic coefficients of covariation (Table 5). No separate tests of significance were conducted.

† Significant at $P < 0.05$ before Bonferroni correction.

that convey high body mass also have lower fertility; (2) high mating ability at young ages is associated with genotypes that have high mating ability at later ages; and (3) genotypes with high early MMA have low rates of senescence.

4. Discussion

(i) Inbreeding depression for male life-history traits

Among the male life-history characters assayed for this study, early and late MMA show larger effects of inbreeding than any other trait. This result and results from previous studies of inbreeding decline in male mating success suggest that there is a substantial genetic load for this character in *Drosophila melanogaster*. Published values of estimates of the inbreeding decline for male reproductive success are shown in Table 7.

The values for the inbreeding decline associated with male mating ability reported by Sharp (1984) were calculated from trials involving either one or three generations of full-sib mating, yielding inbreeding coefficients (F) of 0.25 and 0.5, respectively. A transformation is necessary in order to compare these estimates of inbreeding decline to estimates derived from chromosomal homozygotes (for which $F = 1$ for the chromosome in question). Assuming independent, multiplicative genetic effects, Sharp's values can be used to compute the estimated effects of complete inbreeding by use of the formula:

$$\frac{w_t}{w_0} = \exp(\beta F), \tag{8}$$

where β is the 'inbreeding load', or the regression

coefficient of minus the natural logarithm of fitness on F (Morton *et al.* 1956; Charlesworth & Charlesworth, 1987).

Analogous estimates of the inbreeding load can be computed from the effects of chromosomal homozygosity on male mating scores as measured by Brittnacher (1981), Kosuda (1983), Partridge *et al.* (1985), Miller & Hedrick (1993), and the present analysis. In these calculations, it is assumed that chromosome II accounts for approximately 35% of the *D. melanogaster* genome, and chromosome III accounts for 40% (Charlesworth *et al.* 1992), and the values of β have been adjusted upwards to give per-genome values, again assuming independent, multiplicative effects of loci throughout the genome. The values of β , along with the ages of the males used in different investigations, are reported in Table 7. Calculations of the inbreeding load based on proportions underestimate the load (Greenberg & Crow, 1960; Simmons & Crow, 1977). Therefore, where relative mating ability was originally reported as proportions, I have transformed the proportions to a 'competitive index' $p/(1-p)$, where p is the proportion of experimental males relative to a control, before calculating β . Instances where this transformation was necessary are indicated in Table 7.

The estimates of β for early and late MMA derived from the present analysis fall between the extremes reported by other investigators. Compared to estimates of β for other components of fitness (Table 7, bottom half), the values for early and late MMA are somewhat higher than that reported for egg-to-adult viability, but substantially lower than the value for net fitness. The value of β for net fitness, 3.902, is considerably larger than any others in the table, except that

Table 7. Published estimates of inbreeding decline. F is the inbreeding coefficient of flies used in the analysis and β is the inbreeding load of these flies, calculated from equation (8)

Estimates of inbreeding decline in measures of male mating success						
Character	Inbreeding decline	Type and degree of inbreeding	F	β	Age of males	Source
Male virility	0.437	Homozygosity of chromosome II	1.00	1.641	2 days	Brittnacher (1981)
Male mating activity	0.314	Homozygosity of chromosome II	1.00	1.077	3 days	Kosuda (1983)
Male mating ability	0.118	1 generation of full-sib mating	0.25	0.502	4–7 days	Sharp (1984)
Male mating ability	0.511	3 generations of full-sib mating	0.50	1.431	4–7 days	Sharp (1984)
Male mating ability	0.629†	Homozygosity for chromosome III	1.00	2.478†	3 days	Partridge <i>et al.</i> (1985)
Male mating ability	0.674*	Homozygosity of chromosome II	1.00	3.202*	3 days	Miller & Hedrick (1993)
Male mating ability	0.345†	Homozygosity of chromosome III	1.00	1.058†	3 days	Present analysis
Male mating ability	0.569†	Homozygosity of chromosome III	1.00	2.104†	21 days	Present analysis
				Mean β : 1.365‡		
Estimates of inbreeding decline for fitness components other than mating success						
Character	Inbreeding decline	Type and degree of inbreeding	F	β		Source
Egg-to-adult-viability	0.247	Homozygosity of chromosome III	1.00	0.709		Simmons & Crow (1977)
Net fitness	0.790	Homozygosity of chromosome III	1.00	3.902		Simmons & Crow (1977)

* Calculated from data in the paper, excluding one line that appeared to be semi-sterile (line 17).

† Mating ability index transformed from proportions.

‡ Calculated from β values for flies less than one week old, excluding Miller & Hedrick (1993).

reported by Miller & Hedrick (1993), which may be biased upwards (see below). Most estimates reported in Table 7 are based on assays of male flies less than one week old. The mean value of β for all estimates for young males is 1.63. The mean is 1.37 for estimates from young flies excluding that of Miller & Hedrick (1993).

Estimates of inbreeding decline in male mating ability in the present study and in the other studies summarized in Table 7 all used somewhat different methodologies, and different estimators for scoring mating ability. In particular, Sharp (1984) performed mating trials for outbred flies and for two classes of inbred flies at different times, so that his results may be confounded with environmental differences. Miller & Hedrick (1993) used pre-selected lines for their estimate of the effects of inbreeding. Their selection criteria involved choosing for analysis those homozygous lines that performed poorly in assays of net fitness. The value of β that I have calculated from their results may therefore overestimate the inbreeding decline associated with mating ability in their population. Pendlebury & Kidwell (1974) also evaluate the effect of inbreeding on male mating ability, but their experiment used a very different technique for comparing inbred and outbred fitness, and is not included in Table 7.

The present results and the results of the studies summarized in Table 7 indicate that the inbred load

for MMA is somewhat higher than that for egg-to-adult viability. Of the fitness components analysed in this and previous investigations, none show an inbreeding load which approaches that for net fitness. The fact that the large inbreeding decline for net fitness cannot be accounted for by any single fitness component must mean that no single component is primarily responsible for the decline. From these data, it appears that male mating ability and egg-to-adult viability both contribute substantially to the genetic load for fitness, and are both important components of total fitness in *Drosophila*.

(ii) Dominance of alleles affecting male life-history characters

Estimates of \bar{h} for *D. melanogaster* viability, provided from a number of studies by Mukai and his associates, suggest that mildly deleterious second chromosome mutations have h values of about 0.21, while third chromosome mutations have a somewhat higher value (0.4, estimated from a set of chromosomes that was not screened for inversions) (Mukai, 1972; Mukai & Yamaguchi, 1974; Watanabe *et al.* 1976). These estimates were made from studies of chromosomal heterozygotes, and should be directly comparable to the values of \bar{h} reported in Table 2. Overall, the values of \bar{h} for male fitness components agree well with

estimates obtained for viability by Mukai and his associates, although the sampling errors of the estimates for individual characters are large. Values of \bar{h} for late MMA and mean longevity are somewhat higher than values for the other fitness components included in the present study and in the Mukai *et al.* (1972) study of viability in inversion-free second chromosomes. Among all these estimates, the value of \bar{h} for male fertility is anomalously low. There may be some bias in the estimate of \bar{h} for fertility due to the removal of many sterile and partially sterile lines from the analysis. If highly deleterious alleles are typically recessive, however, any bias due to removal of semi-sterile lines should be in the opposite direction. Alternately, it is possible that fertility is so closely related to total fitness in male flies, that selection has removed most genetic variability except that maintained by overdominance. This interpretation is consistent with the observation that the standing genetic variability for both homozygous and heterozygous lines are low, compared to other fitness components (results for heterozygous lines reported in Hughes, ms. in press), but seems inconsistent with the observation of little or no inbreeding depression for this character.

Although h_1 and h_2 are not significantly different from each other for any of the characters, h_2 is generally larger than h_1 . Perhaps the most important difference between the two estimates is that they are based on different subsets of the experimental lines. While h_2 was calculated from all lines for which data was available, h_1 was calculated from only those heterozygous lines for which both corresponding homozygous lines were available. Because homozygous crosses were generally less productive than heterozygous ones, several homozygous genotypes were typically missing from a given assay. Therefore, only a subset of heterozygous genotypes could be included in the calculation of h_1 . If these heterozygous genotypes represent a non-random subset (with respect to the dominance properties of the segregating alleles), a biased estimate of h would be produced. However, the bias is expected to be in the opposite direction to that observed, unless the viability effects of segregating alleles are generally negatively correlated with their effects on the components of male fitness shown in Table 2. Some evidence for this hypothesis can be deduced from the fact that all estimable genetic correlations involving the fertility character are negative (Table 6). In the assay employed here, the male fertility character includes a component of offspring viability.

Estimates of average dominance for male life-history characters are relevant to the question of the maintenance of genetic variability by overdominance. Overdominant loci have h values that are negative. None of the characters evaluated in this study, except fertility, have estimates of \bar{h} that are less than zero. (Both of the methods of calculating \bar{h} assume that the

frequency of mutant alleles is low. This assumption may not hold for overdominant alleles.) These results do not rule out the possibility that variability at some loci is maintained by overdominance, but they do suggest that alleles at most variable loci are partially recessive or additive in their effects on fitness components. Overall, the results are consistent with the mutation-selection balance model of the maintenance of genetic variation. The values of \bar{h} for most male life-history characters agree well with the values obtained by Mukai for viability. Mukai obtained estimates of 0.35 for newly arisen viability mutations and 0.21 for viability mutations from an equilibrium population. The mean of the \bar{h}_1 estimates (those calculated by the same method as Mukai's estimates) over all the characters in the current study is 0.114 (0.159 if fertility is excluded). The mean over all the variance component estimates (\bar{h}_2) is somewhat higher, 0.250, assuming a value of 0 for fertility.

(iii) Phenotypic and genetic correlations among life-history characters

Phenotypic and genetic correlations between characters in homozygous lines show little correspondence in this study. Of the 14 comparisons that are possible between the entries of Tables 4 and 6, five show a reversal in sign in the two tables, and nine are the same sign in both tables. Only six of the 14 correlations are similar in sign and in magnitude (with a difference less than 0.4) in the two tables. A similar lack of correspondence has been demonstrated between additive genetic correlations (r_A) and r_P (Hughes, ms. under review). These results suggest that not much confidence can be placed in r_P as a valid predictor of r_G for this suite of characters.

This research was completed in partial fulfilment of the requirements of the Ph.D. degree at the University of Chicago. Brian Charlesworth provided valuable guidance and comments throughout the project, and suggested the variance-component method for calculating the average dominance values. Jeanne Altmann, Stevan Arnold, Deborah Charlesworth, Jerry Coyne, and Michael Wade provided advice on the design of the experiments and the analysis of the results. Laurence Mueller suggested using equation (1) to estimate the Gompertz parameters, and two anonymous reviewers made valuable suggestions. Stavroula Assimacopoulos, Darlene Canada, Kevin Dixon, and Kathleen Donohue provided help with many of the experiments. Funding was provided by an N.S.F. Pre-doctoral Fellowship, an N.I.H. Genetics Training Grant to the University of Chicago, and N.I.H. grant AG07748 to B. Charlesworth. Support during the preparation of the manuscript was provided by the Chicago Zoological Society.

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