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Age-Specific Patterns of Genetic Variance in *Drosophila melanogaster*. I. Mortality

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Manuscript received July 12, 1995
Accepted for publication March 1, 1996

ABSTRACT

PETER MEDAWAR proposed that senescence arises from an age-related decline in the force of selection, which allows late-acting deleterious mutations to accumulate. Subsequent workers have suggested that mutation accumulation could produce an age-related increase in additive genetic variance (V_A) for fitness traits, as recently found in *Drosophila melanogaster*. Here we report results from a genetic analysis of mortality in 65,134 *D. melanogaster*. Additive genetic variance for female mortality rates increases from 0.007 in the first week of life to 0.325 by the third week, and then declines to 0.002 by the seventh week. Males show a similar pattern, though total variance is lower than in females. In contrast to a predicted divergence in mortality curves, mortality curves of different genotypes are roughly parallel. Using a three-parameter model, we find significant V_A for the slope and constant term of the curve describing age-specific mortality rates, and also for the rate at which mortality decelerates late in life. These results fail to support a prediction derived from MEDAWAR's "mutation accumulation" theory for the evolution of senescence. However, our results could be consistent with alternative interpretations of evolutionary models of aging.

THE genetic basis of life history traits is of primary interest in the study of evolution, since their component parts, age-specific mortality and fecundity, are directly related to fitness (CHARLESWORTH 1994). An extensive body of theoretical work, using both genetic and optimality approaches, has examined the evolutionary dynamics of life history traits and their underlying genetics (reviewed in ROFF 1992; CHARLESWORTH 1994). At the same time, empirical biologists have studied the genetic basis of life history traits in a wide range of organisms (ROFF 1992; STEARNS 1992). With some exceptions (*e.g.*, ROSE and CHARLESWORTH 1980, 1981; KOSUDA 1985; ENGSTRÖM *et al.* 1989; HUGHES and CHARLESWORTH 1994; HUGHES 1995), genetic studies of life history traits have ignored age-specific effects, focusing on characters such as developmental rate, lifetime fecundity, age at first reproduction, larval viability, or maximum life span. However, to gain a more complete understanding of the evolution of life history traits, we need to examine their genetic basis within the context of age structure. In particular, we need to consider the genetic basis for variation in mortality and fecundity as a function of age.

The importance of age structure to population genetics was first made clear by both FISHER (1930) and HALDANE (1941), who suggested that the strength of selection will be lower in traits expressed at older ages. Some years later, MEDAWAR (1946, 1952) pointed out how an age-related decline in the strength of natural selection could give rise to senescence. Senescence can be defined as a persistent decline in age-specific fitness components of an organism due to internal physiological deterioration (ROSE 1991, p. 20). MEDAWAR noted that a deleterious mutation first expressed late in life is less likely to be selected against than the same deleterious mutation expressed early in life. Due to this declining strength of selection at late age, late-acting deleterious mutations accumulate at a faster rate than early-acting ones. It is this "mutation accumulation" (ROSE and CHARLESWORTH 1980), MEDAWAR argued, that leads to the inevitability of senescence in age-structured populations. CHARLESWORTH (1990) constructed a model to determine the effect of mutation on age-specific survival and fecundity and their genetic variances. After making a series of assumptions about the effects of novel mutations, CHARLESWORTH (1990) found that mutation accumulation can give rise to an age-related increase in additive genetic variance (V_A) for fitness-related traits.

The prediction that V_A increases with age has been tested in *Drosophila melanogaster* with respect to several demographic fitness components, including fecundity, male mating ability and mortality. Workers have estimated genetic variance components for fecundity in flies of different ages (ROSE and CHARLESWORTH 1981; ENGSTRÖM *et al.* 1989) but with contrasting results. ROSE and CHARLESWORTH (1981) found no significant increase in V_A for fecundity. In contrast, in a comparison of 1- and 3-wk-old flies, ENGSTRÖM *et al.* (1989) found a 30-fold increase in V_A for fecundity. Similarly, KOSUDA (1985) and HUGHES (1995) found an age-related increase in variance for male mating ability. Finally, HUGHES and CHARLESWORTH (1994) (hereafter HC)

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examined components of variance for age-specific mortality in *D. melanogaster.* They found a dramatic age-related increase in V_A for age-specific mortality (see also HUGHES 1995), as predicted by CHARLESWORTH's (1990) model of mutation accumulation.

Here we present results from a study in which we estimated genetic variance components for both fecundity and mortality in a mixed-sex cohort of >65,000 same-age *D. melanogaster.* We present a detailed quantitative genetic analysis of mortality rates at different ages, using techniques first developed by MUKAI (1974). In a companion paper (TATAR *et al.* 1996), we examine genetic variance components for fecundity and age-specific patterns of genetic covariance between fecundity and mortality. The fundamental difference between this work and the previous studies mentioned above lies in our use of large numbers of individuals. As we describe in more detail below, small sample sizes in genetic studies of demographic parameters can lead to age-related biases in estimates of variance components.

The primary goal of this work is to examine genetic variance components for mortality and fecundity (TATAR *et al.* 1996) as a test of MEDAWAR's mutation accumulation theory. However, our data shed light on two other questions that are of interest to those studying the genetic basis of aging. First, with these data we can obtain the most accurate estimates to date of the constant term and slope for the Gompertz model (GOMPERTZ 1825) of mortality rate *vs.* age in *D. melanogaster.* And second, recent demographic work has found that late in life mortality rates level off (CAREY *et al.* 1992; CURTSINGER *et al.* 1992; FUKUI *et al.* 1993; VAUPEL *et al.* 1994; KHAZAELI *et al.* 1995a; Khazaeli *et al.* 1996) and may even decline (CAREY *et al.* 1992). Here we provide the first quantitative genetic analysis of decelerating mortality.

MATERIALS AND METHODS

Drosophila genotypes: To estimate genetic variance components for mortality, we used lines derived from a standard balancer chromosome stock *SM1* (FUKUI *et al.* 1996). This stock consists of a second chromosome carrying the dominant mutant *Curly wing* (*Cy*), a recessive lethal sp^2, and multiple inversions to suppress crossing over (DOBZHANSKY and SPASSKY 1953). Females from *SM1* were crossed with males from the wild-type stock *LF350,* a large outbred stock maintained in continuous population cage culture at the University of Minnesota for more than 10 years (WEBER and DIGGINS 1990). The F_1 males from these crosses were backcrossed to *SM1,* and the resultant F_2 offspring crossed within families to create lines with a single wild-type genotype (FUKUI *et al.* 1996) (see DOBZHANSKY and SPASSKY 1953; HUGHES 1995 for schematic illustrations of this procedure).

Of the 53 balancer chromosome lines, nine were discarded because of low viability or fecundity (FUKUI *et al.* 1996). The remaining 44 lines were maintained in either 8-dram vials or half-pint bottles on a 2-wk generation cycle for 12 generations. At this time, we selected 10 lines at random, from which we made intraline crosses to develop stocks that were homozygous wild type for the second chromosome. These stocks were then expanded under controlled larval density through four generations in half-pint milk bottles, at which point at least 4000 newly eclosed individuals could be collected from each line. We isolated virgin males and females under light CO_2 anesthesia from each of the 10 lines and paired them in a 5 × 5 reciprocal, partial diallel cross (North Carolina II cross; COMSTOCK and ROBINSON 1952). Under this design, groups of females from each of the five stocks are paired with groups of males from each of the other five stocks, giving rise to 25 novel genotypes that are heterozygous at the second chromosome. We also performed reciprocal crosses to make a total of 50 genotypes. For each cross, we filled each of 16 milk bottles with ~25 females from one line and 25 males from another. Same-aged offspring (48 hr cohort) from these crosses were used to estimate mortality rates and fecundity.

Culture conditions: Balancer stocks were maintained in half-pint bottles in a constant temperature (24°), constant light walk-in incubator at ~60% relative humidity, and reared on standard agar-yeast-molasses-cornmeal medium with fungicide.

During the demographic assay, flies were housed under the same light and temperature regime but were kept in 3.8-l clear plastic cages designed specifically for estimating mortality rates (FUKUI and KIRSCHER 1992). These cages are inverted plastic jugs that have a 10 × 10 cm screen of mosquito netting, a gusseted aperture near the bottom for instrument access and a screen tautly fastened across the opening of the jug. The cage is placed atop a dish, fashioned from the lid of the jug, filled with standard medium that is covered with a single layer of absorbent gauze (USP type III 28/24 gauze, "Parkdale," Professional Medical Products). The cage and dish are held together by a pair of longitudinal rubber bands. Through the screen and gauze, flies readily eat from and lay eggs on the medium. Each day the food dish was removed, the exterior of the screen was cleaned and dead flies were removed from the interior of the cage by suction. New food was provided four times per week, and screens were replaced in the fourth week and approximately every 10 days thereafter.

Experimental setup: From each reciprocal cross we collected at least 1300 newly eclosed flies in a 48-hr period without anesthesia. Flies from each cross were weighed to estimate the approximate number of individuals and then divided between two cages, with ~650 males and females per mixed-sex cage. There were 100 cages altogether, with an average of 650 flies per cage, for a total of 65,134 flies in the experiment. For purposes of genetic analysis, our sample size consists of 100 cages. Within each cage, we use large numbers of flies (genetically identical at the second chromosome) to reduce sampling error in our mortality estimates as much as possible. Flies within cages are essentially clonal replicates with respect to the genetic analysis. In this experiment, a single cage of flies is treated as an individual, with an associated mortality rate at each age. Mortality rate is an aggregate statistic averaged over many individuals, so observed environmental variances will necessarily be underestimates of the true, among-individual environmental variance.

Mortality estimation: To estimate mortality rates, every day we removed, sexed and counted the dead flies in each cage. Cages were kept outside of the incubator for no more than 1 hr per day. For each sex within each cage, we can assign a value d_x, the number of deaths at age x. After the last fly in a cage had died, we calculated the number of flies in the initial cohort (N_0) for that cage as the sum of the deaths over all ages. From N_0 and d_x, we were then able to calculate the number alive at any age, N_x. The probability of surviving from age x to age $x + 1$, $P_x = N_{x+1}/N_x$. The mortality rate, μ_x, is defined as

$$\mu_x \approx -\ln (P_x) \quad (1)$$

(ELANDT-JOHNSON and JOHNSON 1980).

Variance component analysis: Genetic variance components were estimated for the natural logarithm of mortality rate (*i.e.*, ln $[\mu_x]$). We also determined variance components for parameters from two separate mortality models that describe the trajectory of mortality as a function of age. The balancer-chromosome design we used here reveals genetic variance for the second chromosome, which accounts for ~40% of the genome (ASHBURNER 1989).

To estimate genetic variance components, we used *Quercus*, a package of programs for maximum likelihood (ML) analysis of quantitative genetic data, developed by R. and F. SHAW (1992, 1994). We used the restricted maximum likelihood (REML) option (SHAW 1987) of *Quercus' nf6.p*. The program provides unbiased estimates for genetic and nongenetic components of variance, including additive (V_A), dominance (V_D) and environmental (V_E) variance, and maternal effects (V_M). There are many advantages to using REML, rather than ANOVA, to estimate variance components. In particular, REML does not depend on balanced design, nor is it limited by the genetic relationships in the data (SHAW 1987; see also MEYER and THOMPSON 1984). These models do assume, however, that the data are normally distributed. It is not yet clear how the significance tests would be influenced by deviations from normality, though SHAW *et al.* (1996) provide at least one example where violation of normality did not compromise the tests.

A related program, *pcrfl*, compares genetic variance-covariance matrices for separate populations. We used this program to test whether V_A for mortality rate differed significantly between any two age classes. Results from this program must be interpreted with caution. The program assumes that the two populations being compared are genetically independent. In our case, we compare the same population at two different ages, so they are not genetically independent.

We also calculated variance components for mean longevity, or age of individual death. These data consist of individual measures on 34,358 females and 30,776 males, instead of means within cages. Individuals within cages are treated as full sibs of fully inbred, unrelated parents. To analyze variance components for life span, we used a modified version of *nf6.p* that facilitates maximum likelihood analysis with very large data sets.

The parental lines are homozygous at the second chromosome. Accordingly, in our analysis with *nf6.p* we estimated variance components assuming an inbreeding coefficient of 1.0 in parents. Under this assumption, the estimate of V_A from *Quercus* is divided by a factor of 2 (*e.g.*, MUKAI *et al.* 1974).

As with a previous test of genetic variance for mortality rate (HC), our analytical approach assumes that mortality estimates for different age classes are independent. Strictly speaking, this may not be true if the mortality rate at one age is genetically correlated with that at later ages (see TATAR *et al.* 1996). However, as we point out in the discussion below, a comprehensive solution to this problem lies beyond the scope of existing statistical models.

Additive variance for mortality rate as a function of age: Our central focus here is on the analysis of genetic variance components for mortality rate, μ_x. Males and females were analyzed separately. Weekly mortality estimates were determined following Equation 1 and were log-transformed to normalize the variance (see RESULTS, below). We used weekly estimates, rather than daily estimates, to reduce the effects of sampling error. When we log-transform the data, if there were no deaths in a cage during an entire week, then log (μ_x) = log(0) was undefined. In this case, we treated the cage as a missing value. Similarly, once all individuals in a cage had died, that cage was treated as a missing value. In addition to logarithmic transformations, we also transformed our data using a standardization similar to that performed by HC. This standardization divides mortality for the *i*th genotype at age x ($\mu_{x,i}$) by the mean for that age among all genotypes, such that the average standardized mortality rate for each age, $\bar{\mu}_x = 1$. To test for departure from normality for both the logarithmic and standardization transformations, we used a SHAPIRO-WILK's test (SHAPIRO and WILK 1965) on the residuals from the genetic analysis.

To determine whether there were significant changes in V_A among age classes, we compared estimates of V_A for mortality between pairs of age classes using the program *pcrfl*, as described above.

We used the log-likelihood ratio test to determine statistical significance for both *nf6.p* (H_0: $V_A = 0$) and *pcrfl* (H_0: $V_{A(\text{age } i)} = V_{A(\text{age } j)}$). To do this, we calculated log-likelihoods (LL) for the model, with the genetic variance component whose significance we wish to determine either included (LL_{inc}) or excluded (LL_{excl}) from the model. The two-tailed P value was determined from the log-likelihood ratio, $2 \cdot (LL_{inc} - LL_{excl})$, which is distributed as a chi-squared statistic with one degree of freedom (SHAW 1987) when parameters are tested one at a time.

Slope and constant parameters for mortality curves: A plot of ln(μ_x) *vs.* age x describes the mortality curve for a given cohort. Many studies, relying on relatively small sample sizes (tens or hundreds of individuals), have found that mortality rates increase exponentially with age (*e.g.*, COMFORT 1979; FINCH 1990; FINCH *et al.* 1990; PROMISLOW 1991; TATAR *et al.* 1993). In this case, the mortality curve is well described by the Gompertz equation, $\mu_x = Ae^{Bx}$, where A and B are variables describing the age-independent constant term and slope of the line, respectively. Taking the logarithm of both sides, we see that

$$\log(\mu_x) = \log(A) + B \cdot x. \quad (2)$$

Previous studies have estimated values for A and B in different genotypes based on small cohort sizes (tens of individuals), which can lead to consistent bias in parameter estimates (WITTEN 1994; MUELLER *et al.* 1995). To avoid this problem, we used much larger sample sizes and estimated the Gompertz parameters for males and females in each of 100 cages, based on daily censuses, using maximum likelihood (FUKUI *et al.* 1993, Gauss system version 2.1).

Use of an inappropriate mortality model may also affect the accuracy of the estimates of A and B. In fact, nonlinear patterns of mortality may be common: researchers have found a marked deceleration in the rate of increase in mortality late in life (ECONOMOS 1982; ABRAMS 1991; CAREY *et al.* 1992; CURTSINGER *et al.* 1992; FUKUI *et al.* 1993; VAUPEL *et al.* 1994; KHAZAELI *et al.* 1995a; KHAZAELI *et al.* 1996). A previous study of mortality rates in Drosophila tested several alternatives to the Gompertz, including two-stage Gompertz, Weibull and logistic models, and found the logistic model provided a superior fit to the data (FUKUI *et al.* 1996). Thus, to correct for departure from Gompertz-like mortality, we used ML to fit the logistic model (VAUPEL 1990), which includes a term, S, for deceleration in mortality,

$$\mu_x = \frac{Ae^{Bx}}{1 + \frac{S \cdot A}{B}(e^{Bx} - 1)} \quad (3)$$

This model has the property that for $A, B > 0$, and $0 < S < B/A$, $(d/dx)[\ln(\mu_x)] > 0$ and $(d^2/dx^2[\ln(\mu_x)] < 0$. The Gompertz model is a special case of the logistic model when $S = 0$. As S increases, the rate of increase in mortality deceler-

TABLE 1

Shapiro-Wilk's test for normality of residuals

	Males		Females	
Age (wk)	Standardized	Log	Standardized	Log
1	0.714***	0.962*	0.719***	0.945**
2	0.851***	0.972 (ns)	0.941***	0.965 (ns)
3	0.954*	0.946**	0.823***	0.972 (ns)
4	0.918***	0.959*	0.827***	0.975 (ns)
5	0.915***	0.978 (ns)	0.919***	0.969 (ns)
6	0.961*	0.971 (ns)	0.957*	0.974 (ns)
7	0.910***	0.990 (ns)	0.982 (ns)	0.973 (ns)
8	0.897***	0.992 (ns)	—	—

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Smaller P values indicate greater departure from normality, based on the SHAPIRO-WILK's test (SHAPIRO and WILK 1965). Tests for normality were performed on the residual values determined from the genetic variance component analysis. Residuals from the standardized analysis depart significantly from normality in all but one case, while residuals from the log-transformed case are generally normal.

ates. For the curve-fitting procedure, which was based on daily census, we omitted the first 10 days from our data, after which time the data appear to increase exponentially with age (see TATAR and CAREY 1994b).

Phenotypic and genetic analysis of mortality curves: In their study of *D. melanogaster*, HC reported that Gompertz curves, estimated by linear regression, diverge with age. They base this claim, in part, on the observation that the additive coefficient of variation (CV_A) for the slope of the Gompertz line (B) was significantly different from zero, while CV_A for the constant (A) was not.

We repeated this estimate with our own data but in a slightly different manner. First, to determine whether or not the mortality curves diverge, one can plot the curves superimposed on one another. We did this by plotting the relationship between log-transformed mortality (3 day running average) as a function of age, without assuming any particular underlying mortality model. Where mortality rates equaled zero and were undefined under the log-transformation, we treated those entries as missing values. Second, we determined the V_A for A, B and S, as described in (2) and (3).

RESULTS

Mortality rates: Among age classes, the variance in mortality rate shows a strong dependence on the mean mortality (females: $\sigma^2_{[\mu]}$ *vs.* μ: Pearson $r = 0.996$, $n = 7$, $P < 0.0001$; males: $\sigma^2_{[\mu]}$ *vs.* μ: Pearson $r = 0.99$, $n = 8$, $P < 0.0001$). We need to correct for this because mortality increases with age, and we are interested in determining whether variance components increase with age, independent of any age-related changes in mortality rate. A logarithmic transform normalizes the data but forces us to omit cages in any period for which $\mu_x = 0$, where the transformation is undefined. For the females, we omitted 12 cages at week 1, two at week 2, and one at week 6, for a total of 15/683 cage weeks or 2.2%. For the males we omitted 12 at week 1, six at week 2, and one at week 3, for a total of 19/798 cage-weeks or 2.4%. HC suggested using standardized mortality (defined above), which enables us to use cages where $\mu_x = 0$. This effectively removes the mean-variance relationship, as all age classes have mean mortality $\bar{\mu}_x = 1$. The logarithmic transformation does not remove the dependence of the variance on the mean, but it does switch the sign of the relationship, such that variance is lower at higher values of μ for both females ($\sigma^2_{[\log \mu]}$ *vs.* $\log \mu$: Pearson $r = -0.86$, $n = 7$, $P = 0.014$) and males ($\sigma^2_{[\log \mu]}$ *vs.* $\log \mu$: Pearson $r = -0.94$, $n = 8$, $P = 0.0006$).

However, with either the logarithmic or standardized transformation, the variance of mortality rate decreases with age, and at similar rates. The advantage of the logarithmic transformation *vs.* the standardization lies in the ability of the logarithmic transformation to normalize the variance within age classes. The standardized data tend to be skewed to the right at early ages due to the existence of zero values, which alone are not transformed by the standardization ($0/\bar{\mu} = 0$). The log-transformed data do not generally depart from normality (Table 1). Based on an analysis of residuals for departure from normality, we found that all but one of 14 samples of the standardized data were significantly different from normal. Residuals of the log-transformed data were significantly nonnormal in only four cases, and in general the departure from normality was less significant for the log-transformed data than it was for the standardized data. For this reason, we restrict our genetic analysis to log-transformed mortality data.

Our analysis of genetic variance components for log-transformed mortality rates shows a pattern strikingly different from the predicted monotonic increase in V_A with age. Additive genetic variance for log mortality rate increases in both males and females over the first weeks and then decreases from middle to the late ages (Figure 1). Estimates of V_A at middle ages are significantly greater than zero after correcting for multiple comparisons (DUNN-SIDÁK test, SOKAL and ROHLF 1995) (Figure 1).

We used the *pcrf1* model in *Quercus* to test for signifi-

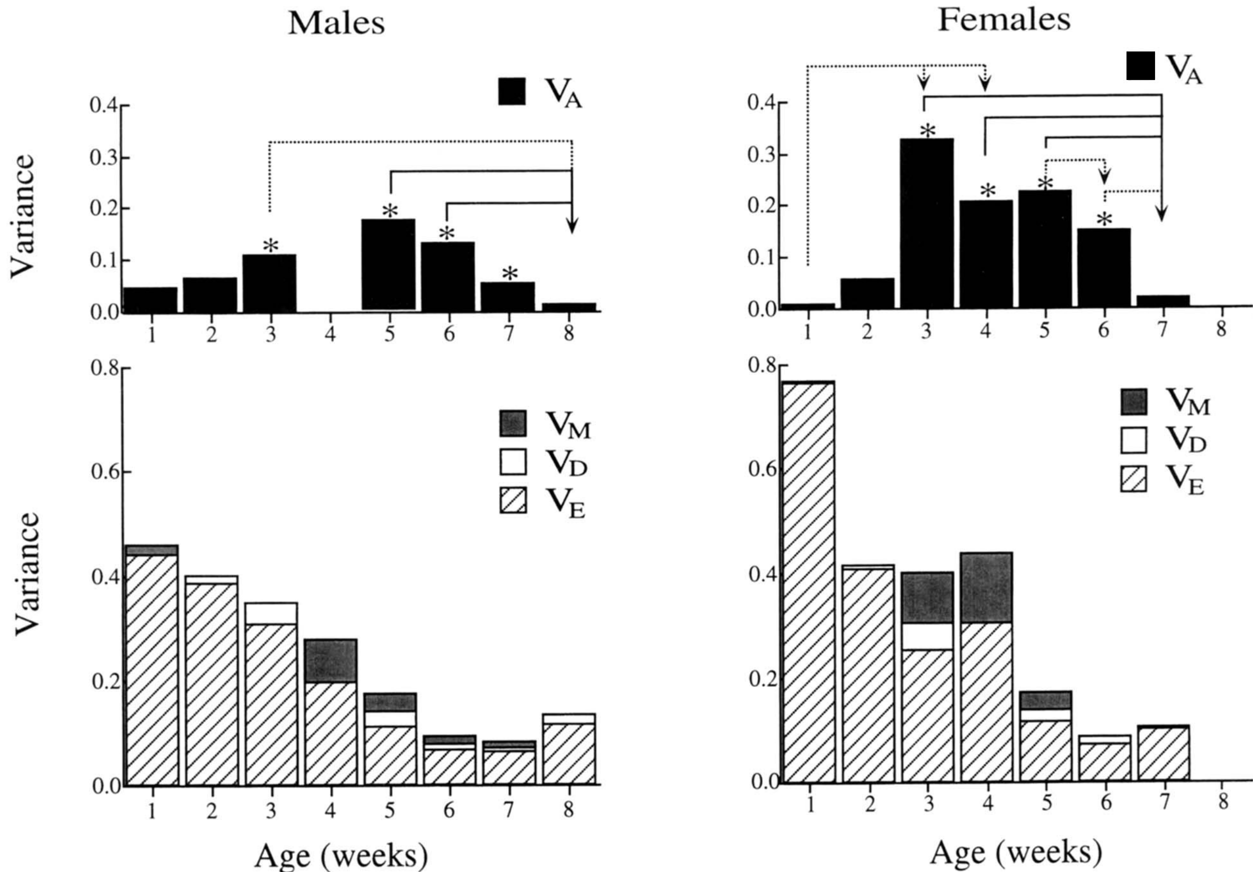

FIGURE 1.—Age-specific patterns of genetic variance components for $\log_{10}$ mortality rate in males and females by week. Genetic variance components were estimated using maximum likelihood (described in text). The upper figures show measures for additive variance (V_A), while the lower figures show variance due to dominance (V_D), environment (V_E) and maternal effects (V_M). *, V_A is significantly greater than zero ($P < 0.005$, except for male class 3, where $P < 0.05$). After DUNN-SIDÁK correction for multiple comparisons (SOKAL and ROHLF 1995), all asterisked tests of $V_A > 0$ remain significant except male class 3. Lines connecting points indicate significant differences (—, $P < 0.05$) or marginally significant differences (··· $0.1 > P > 0.05$) before correction for multiple comparison. After correction, none are significant. However, at least for females, the distribution of observed χ^2 values is significantly greater than by chance ($P < 0.0001$, see text).

cant differences in V_A between age classes. The observed differences among age classes are statistically significant (see Figure 1). However, after correcting for multiple comparisons, the pattern is less clear. For example, between age classes in females, nine of 21 comparisons (43%) are either significant ($P < 0.05$) or nearly so ($P < 0.1$). After employing the DUNN-SIDÁK correction for multiple comparisons (SOKAL and ROHLF 1995), none remains statistically significant. We must interpret any age-related changes in variance components for mortality with caution.

Males and females differ somewhat in the relationship between V_A for mortality rate and age. In females, the pattern of increase and subsequent decline in V_A is relatively clear (Figure 1, females). In males, there is a less obvious pattern of increase and decline because there is no significant variance for mortality during the fourth week (Figure 1, males). During this week, cages were cleaned and all screens were changed for the first time. On the basis of visual inspection of the raw data, the females do not appear to have been affected by this procedure, but the males show a sudden decline in mortality rates, which lasts several days. This decline was independent of genotype and appears to have obscured the underlying genetic variance components for mortality at this age.

Mortality curves: If genetic variance for mortality rates increases monotonically with age, we expect mortality curves among different genotypes to diverge. Figure 2 shows the relationship between the log of mortality (3-day running average) *vs.* age for the combined data from each of 25 genotypes, with the reciprocal crosses pooled, for males and females separately. Each line is based on age at death for an average of 1231 ± 84 males (mean ± SD) or 1374 ± 76 females per genotype. For both males and females, the mortality curves do not diverge at late ages.

HC found that mortality curves diverged with age. The difference between our results and those of HC may be due, in part, to the transformation that they used. If mortality rates follow a Gompertz-like trajectory, then mortality will increase linearly when log trans-

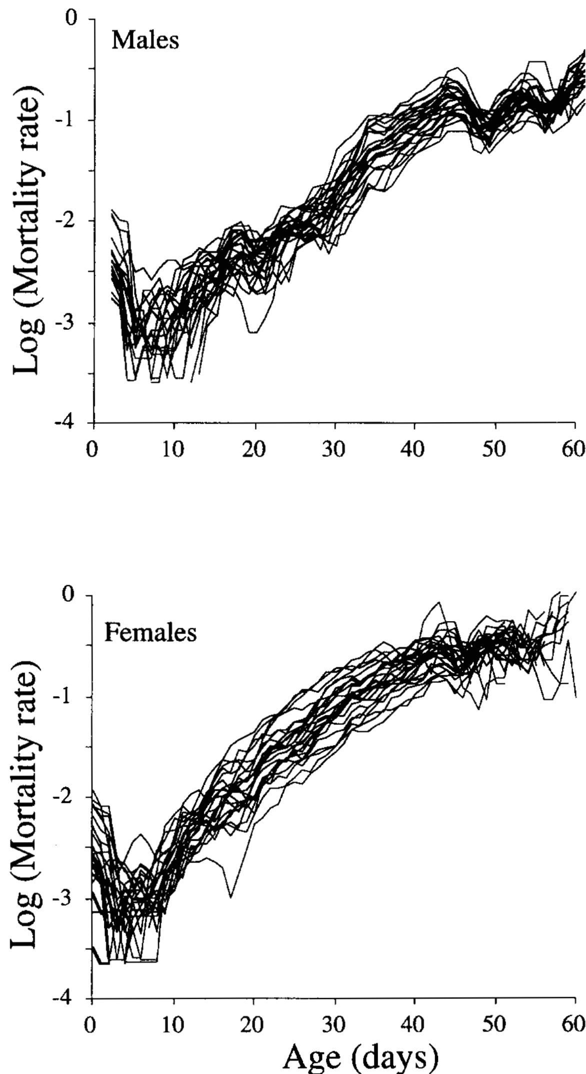

FIGURE 2.—Mortality rate *vs.* age, plotted on a $\log_{10}$ scale, for 25 genotypes for both males and females. For this figure, we pooled data from the reciprocal crosses and from each of two replicate cages within each reciprocal cross to give a total of 25 curves for each sex. Each of the 25 curves is based on an average of 1231 individual flies for males and 1374 individual flies for females. Note that the curves do not diverge and appear to level off in both males and females late in life.

formed. To avoid the situation of $\mu_x = 0$, where $\ln(\mu_x)$ is undefined, HC used a $\ln(\mu_x + 1)$ transformation. However, when this is done, lines that are actually parallel on a log-linear scale become curvilinear and appear to diverge.

To illustrate the confounding effects of the $\ln(\mu_x + 1)$ transformation, consider the following. When mortality rate is log-transformed, the slope of the Gompertz curve is age-independent. When mortality rate is transformed using $\ln(\mu + 1)$, the slope is given by

$$\frac{d}{dx}[\ln(\mu_x + 1)] = \frac{AB \exp^{Bx}}{A \exp^{Bx} + 1}, \qquad (4)$$

which is an increasing function of age, x, for $A, B > 0$. Given that $A \ll 1$, when age $x = 0$, from (2) we have

$$\ln(\mu_0 + 1) = \ln(A + 1) \approx 0. \qquad (5)$$

Thus, using this transformation, at age 0 the lines will converge to $\ln(1) = 0$, even if there is variation for A. Linear regression lines fitted to these data will diverge on this $\ln(\mu + 1)$ scale, even if all lines have identical slope parameters, B, in the Gompertz equation.

Fitting models to mortality rates: Previous studies have found significant additive genetic variance for the slope of the Gompertz curve (2) but not for the constant term (HC; TATAR and CAREY 1994a; but see CURTSINGER *et al.* 1992, who find significant genotypic variation for the constant term). On this basis, HC argue that variance for mortality increases with age. As with previous findings, when we fit the Gompertz model to our data, we find significant genetic variance for the slope in both sexes but only for the constant term among males (females: $0.1 > P > 0.05$) (Table 2). However, the accuracy of these results rests on the assumption that mortality curves follow a Gompertz-like trajectory. We fit our data to the logistic model (3) and obtained a significantly better fit for 80% of the female samples and 88% of the male samples, using a log-likelihood ratio test to compare the goodness-of-fit of these nested models. Using (3), we found highly significant genetic variance for both slope and constant term in males and females (Table 2). Heritable variation in life span may be due not only to genetic variability in rates of senescence (HC), but also to variation in baseline mortality rates. Furthermore, our analysis shows significant V_A for deceleration of the mortality curve in males, and suggestive values of V_A in females ($0.1 > P > 0.05$) (Table 2).

Mean life span: Narrow-sense heritability for mean life span in females was $3.6 \pm 1.5\%$ (mean $\pm$ SD) and in males was $2.6 \pm 1\%$. If we assume that the genetic effects at the second chromosome on mortality rates account for 40% of the genome-wide genetic effects on mortality rates (ASHBURNER 1989), total narrow-sense heritability for female and male life span is ~9 and 6.5%, respectively. Estimates of narrow-sense heritability for life-history traits typically range from 3 to 15% (MOUSSEAU 1987; ROFF and MOUSSEAU 1987). In MATERIALS AND METHODS, we noted that estimates of V_E for age-specific mortality rates are underestimates of the true V_E. Variance components for life span, however, which are based on individual ages at death within each cage, provide an accurate estimate of environmental variance. In this case, V_E is between 25 and 30 times the value of V_A.

DISCUSSION

An explicit model of MEDAWAR's mutation accumulation hypothesis for the evolution of senescence predicts that genetic variance for mortality should increase with age (CHARLESWORTH 1990). Only one previous study

TABLE 2

Additive variance for mortality curve parameters

Model parameter	Males Mean	Males V_A	Females Mean	Females V_A
Gompertz (Equation 6)				
Intercept (ln[*A*])	−6.63	0.140**	−6.58	0.289†
Slope (*B*)	0.101	9.20×10^{-5}**	0.126	2.57×10^{-4}**
Logistic (Equation 7)				
Intercept (ln[*A*])	−8.98	0.747**	−8.16	0.785**
Slope (*B*)	0.186	0.0016**	0.199	0.0026**
Deceleration	0.862	0.0268*	0.673	0.024†

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$.

has examined genetic variance components for mortality (HUGHES and CHARLESWORTH 1994; HC). From their study of age-specific variance components in *D. melanogaster*, HC concluded that (1) mortality curves for different genotypes become increasingly divergent with age, (2) there is significant genetic variance for the slope but not for the constant term of the Gompertz plots of mortality, and (3) additive variance for mortality increases monotonically with age. Our results, based on substantially larger sample sizes, differ in each of these three conclusions. First, in our study mortality curves among genotypes did not diverge. Second, while rates of senescence differed significantly among genotypes, baseline mortality (the constant term of the Gompertz curve) also differed among genotypes. Third, variance components for mortality did, indeed, increase with age, but this increase occurred only during the first half of life, after which there was a marked decline in variance. Our confidence in the statistical robustness of this last result is hindered by the fact that no statistical model exists to account for the distribution of error variances within and among cohorts and between age classes. If true, the observed decline is an unprecedented finding that is not predicted by existing models for the evolution of senescence. Taken together, these three findings fail to support the predictions that have previously been put forth as critical tests of MEDAWAR's mutation accumulation theory (ROSE and CHARLESWORTH 1981; CHARLESWORTH 1990).

The standard alternative model, antagonistic pleiotropy (WILLIAMS 1957; ROSE 1991), is not supported by our data (but see TATAR *et al.* 1996). For antagonistic pleiotropic alleles to maintain variation for fitness-related traits, substantial dominance variance is needed (CURTSINGER *et al.* 1994; HUGHES 1995). We find no significant dominance variance for mortality rates (Figure 1) (see also HUGHES 1995).

Existing interpretations of the mutation accumulation model of aging do not explain the age-related increase and subsequent decline in additive variance observed here. They may also not allow us to distinguish between the antagonistic pleiotropy and mutation accumulation models unequivocally (see also PROMISLOW and TATAR 1994). Predictions about the age-specificity of genetic variance components for both mutation accumulation and antagonistic pleiotropy depend on several important but untested assumptions. In particular, we need to know the role that age plays in the mutation process. Will mutations with age-specific effects occur with equal likelihood across all ages? Do late-acting deleterious mutations act at a single age, or are they expressed at all ages subsequent to the age of onset? Is the strength of the effects of a given mutation dependent on the age at which it acts? We suggest that under some sets of these assumptions, the genetic variance components may increase with age under antagonistic pleiotropy or may fail to increase under mutation accumulation. Clearly, one cannot reject mutation accumulation or antagonistic pleiotropy on this basis. Empirical work on age-specific rates of mutation is needed to facilitate development of new and more biologically realistic theoretical models of senescence.

Why genetic variance for mortality declines with age: Perhaps the most surprising result in this study is our finding that V_A for mortality rates declines at later ages in both males and females. There are several possible explanations, both genetic and nongenetic, for this pattern.

First, the patterns we observed may have been due to temporal changes in the laboratory environment. In HUGHES and CHARLESWORTH's study of genetic variance components for age-specific mortality rates, they found substantial differences in the amount of genetic variation among blocks. To factor out this effect in future studies, control lines could be used to estimate genetic variance components for mortality at specific age classes for the duration of the experiment.

Second, our results may have been influenced by costs of reproduction. Flies in this study were maintained in mixed-sex cages, able to mate and reproduce freely. Mixed-sex cohorts allow us to estimate genetic variance for traits in the presence of sexual competition (HOULE *et al.* 1994) and so may better reflect the patterns found in a natural environment. But mixed-sex

cohorts may not allow us to distinguish reproductive effects from direct mutational effects on age-specific mortality. If variation among genotypes in mortality is due to variation in reproduction, then at late ages, when few genotypes are reproductively active (TATAR *et al.* 1996), variation in mortality may decline.

Costs of reproduction may also account for the approximately twofold increase in V_A in females relative to males. If costs of reproduction are higher in females than in males, and if there is significant genetic variance for reproduction, then we might expect females to show greater genetic variance for mortality than males.

Third, theoretical and experimental work has shown that within-cohort variation in quality (demographic heterogeneity) can lead to departures from exponential Gompertz-like mortality (VAUPEL and YASHIN 1985; KHAZAELI *et al.* 1995b). Within a group of genetically identical individuals, there is a great deal of variation for age at death, due in part to environmentally induced variation in quality. Under certain circumstances, this heterogeneity can give rise to age-related trends in variance similar to those that we observed (*e.g.*, VAUPEL and YASHIN 1985; PROMISLOW, unpublished results). Unfortunately, at present there are no experimental methods for determining the degree of demographic heterogeneity in a population with respect to mortality rate (but see KHAZAELI *et al.* 1995b).

Fourth, as we pointed out in our introduction, insufficient sample size may bias observations, independent of any age-specific change in true V_A. Although we have used the largest sample size to date (over 65,000 flies), at extreme ages our data may still suffer from sampling error. At early ages, few deaths will be observed because mortality rates are low, while at late ages few deaths will be observed because few individuals are left. With very few deaths among all genotypes, we will systematically underestimate genetic variance. To provide a definitive statistical test of the effect of sampling error on estimates of V_A at different ages, we need a model that incorporates the distribution of error variances for mortality within cohorts (binomial or beta-binomial) and between cohorts (log-normal), and also incorporates the age-related change in mean mortality rates. Work is currently underway to develop such a model (F. SHAW, University of Minnesota, personal communication).

Finally, the decline in V_A for mortality may reflect a true decline in genetic variance for fitness traits at late ages. If the decline is biologically real, what genetic mechanism could lead to this decline in variance? We offer the provisional suggestion here that such a decline might be expected if late-acting deleterious mutations are expressed at all ages subsequent to the age of onset.

Existing models of mutation accumulation (*e.g.*, CHARLESWORTH 1990) assume that genotypes differ with respect to the number of alleles with deleterious effects that are expressed at any given age. By contrast, here we suggest that genotypic differences in mortality rates may be due to variation in gene regulation and expression (see, *e.g.*, FINCH 1990, pp. 385–388), and that one or a few loci may be responsible for this variation. If mechanisms of gene regulation break down with age so that at very late ages gene regulation in all genotypes is equally poor, then there may be no genetic variance for mortality late in life. Further modeling efforts are necessary to test this idea.

Gompertz models and the genetic basis of decelerating mortality rates: As with previous studies of mortality rates (HC; TATAR and CAREY 1994a; HUGHES 1995), we found significant genetic variance for the slope of the Gompertz equation but only weak evidence for a genetic basis to the constant term. However, our results suggest that this finding may be due to the failure of the Gompertz model as an adequate predictor for changes in mortality rate with age. As Figure 2 shows, the mortality curves for the population observed here differ from the straight line on a log-linear plot that is predicted by the Gompertz equation (FUKUI *et al.* 1996). With a more detailed, three-parameter model such as that given in (3), we find significant V_A for both slope and for the constant term in both sexes. (Note that the three-parameter model points to significant V_A for baseline mortality, while our direct genetic analysis on mortality during the first week found that V_A is not significantly different from zero. The constant term, A, estimates the elevation of the lines throughout the life span and not just at the intercept.) This result, together with visual inspection of age-trajectory of mortality rates (Figure 2), suggests that mortality curves do not diverge with age.

Several recent studies, including work on Caenorhabditis (VAUPEL *et al.* 1994), Drosophila (CURTSINGER *et al.* 1992; FUKUI *et al.* 1993; KHAZAELI *et al.* 1995a, 1996) and the medfly Ceratitis (CAREY *et al.* 1992) show that mortality rates in laboratory populations may increase exponentially over part of the lifespan but then decelerate at later ages. This may even be true for human populations (ECONOMOS 1982; KANNISTO *et al.* 1994). Although some have argued that mortality rates may decelerate due to a decline in density with age (GRAVES and MUELLER 1993; NUSBAUM *et al.* 1993), this explanation is unlikely. The effect occurs among individually housed flies (CAREY *et al.* 1992) and among flies kept at constant density by supplementation with marked individuals (KHAZAELI *et al.* 1996) (see also CURTSINGER 1994, 1995; KHAZAELI *et al.* 1995a). Alternatively, the deceleration could be due to demographic heterogeneity, as discussed above (VAUPEL and YASHIN 1985). Finally, ABRAMS and LUDWIG (1995) used theoretical models to suggest that non-Gompertz mortality may have an underlying biological basis.

Our study adds the important observation that there is a genetic component to the rate at which mortality decelerates. VAUPEL and YASHIN (1985) demonstrate that mortality rates would decelerate if individuals

within a population are heterogeneous with respect to the mortality trajectory that they follow. The greater the heterogeneity, the greater the rate of deceleration. Thus, the genetic variance for deceleration that we observed could arise if genotypes differed in the amount of within-cohort heterogeneity. Similarly, a genetic component for deceleration could arise if genotypes differed in costs of reproduction (*e.g.*, ABRAMS and LUDWIG 1995). However, in our genetic analysis of the logistic model (3), values of S are negatively correlated with A and positively correlated with B (data not shown), though this could be due to sampling error covariance. The variance component estimates for S are not necessarily independent of those for A and B, and further work is needed to develop statistical models that will estimate genetic variance components for S independent of values for A and B.

The way to a definitive answer: There are two major issues that need to be resolved before we can definitively answer the question of how genetic variance components for mortality change with age. First, to obtain unbiased estimates for genetic variance for mortality rates, we need a maximum likelihood model that will account for the fact that the trait is binomially or betabinomially distributed within cohorts, log-normally distributed among genotypes, and increases approximately exponentially with age at least over the first half of the life span. Until we have achieved this, variance component estimates for mortality rates must be interpreted with due caution.

Second, we point out above that the predictions that arise from mutation accumulation or antagonistic pleiotropy theories for the evolution of senescence may depend largely on our assumptions about the effects of deleterious mutations at different ages. We suggest that more specific evolutionary models for aging need to be developed, including ones that examine assumptions about the age-specificity of both rates and effects of polygenic mutations, and that offer mutually exclusive, testable hypotheses. We believe that these alternative models will likely integrate the insights of MEDAWAR and WILLIAMS with the details of reproductive behavior (*e.g.*, ABRAMS and LUDWIG 1995; TATAR and CAREY 1995) or genetic mechanisms (*e.g.*, MURRAY 1990).

Throughout the course of this work, generous assistance on conceptual, experimental and statistical issues has been provided by many colleagues, including P. ABRAMS, J. CAREY, B. CHARLESWORTH, G. ENGSTRÖM, C. GEYER, H. FUKUI, P. HARVEY, K. HUGHES, M. LYNCH, L. PARTRIDGE, S. PLETCHER, F. SHAW, R. SHAW, M. TURELLI, J. VAUPEL, two anonymous reviewers, and Dr. DOGMA. For technical assistance, we thank J. ASHLEY, K. BRAAS, H. FUKUI, K. KELLY, A. KIRSCHER, C. MISIAK, S. PLETCHER, A. RESLER, N. STAUS, and D. WOODWARD. Special thanks to RUTH and FRANK SHAW, whose wise and patient advice helped to steer us clear of many pitfalls. This work was supported by National Institutes of Health grants AG-0871 and AG-11722 to J.W.C.

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Communicating editor: M. LYNCH