The Evolution of Age-Specific Mortality Rates in *Drosophila melanogaster*: Genetic Divergence Among Unselected Lines

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ABSTRACT

Age-specific effects of spontaneous mutations on mortality rates in Drosophila are inferred from three large demographic experiments. Data were collected from inbred lines that were allowed to accumulate spontaneous mutations for 10, 19, and 47 generations. Estimates of age-specific mutational variance for mortality were based on data from all three experiments, totalling ~225,000 flies, using a model developed for genetic analysis of age-dependent traits (the character process model). Both within- and among-generation analyses suggest that the input of genetic variance is greater for early life mortality rates than for mortality at older ages. In females, age-specific mutational variances ranged over an order of magnitude from 5.96×10^{-3} at 2 wk posteclosion to 0.02×10^{-3} at 7 wk. The male data show a similar pattern. Age-specific genetic variances were substantially less at generation 47 than at generation 19—an unexplained observation that is likely due to block effects. Mutational correlations among mortality rates at different ages tend to increase with the accumulation of new mutations. Comparison of the mutation-accumulation lines at generations 19 and 47 with their respective control lines suggests little age-specific mutational bias.

THE properties of spontaneous mutations figure prominently in many areas of evolutionary theory. The rate of input in genetic variance due to new mutations (the mutational variance) is a fundamental parameter of models that consider the amount of genetic variation expected to be maintained for that character in natural populations (Barton and Turelli 1989; Kondrashov and Turelli 1992; Houle *et al.* 1996) and in models that predict the long-term response of the trait to directional selection (Hill 1982; Keightley and Hill 1987). For fitness-related characters, the effects of the accumulation of new mutations on the mean value of the trait (and pleiotropically on fitness) are important for theory concerning, among others, the evolution of sex (Kondrashov 1988), the evolutionary dynamics of sexually selected characters (Pomiankowski *et al.* 1991), and the conservation of small populations (Lynch *et al.* 1995). Moreover, whether mutational properties show some form of age specificity is important to many aspects of life-history theory, especially the evolution of senescence (Charlesworth 1994; Pletcher and Curtsinger 1998; Promislow and Tatar 1998).

In a hallmark article, Mukai (1964) reported the first estimates of the rates and effects of spontaneous mutations on viability in *Drosophila melanogaster*. Since then, the number of published reports investigating the properties of new mutations has been accelerating (Lynch 1988b; Mackay *et al.* 1992; Fernandez and Lopez-Fanjul 1996; Keightley and Caballero 1997). We now have a good deal of information on the mutational variance for several traits in Drosophila (Mackay *et al.* 1992; Houle *et al.* 1994; Pletcher *et al.* 1998) and for a variety of characters in other species (Lynch 1988b; Kibota and Lynch 1996; Keightley and Caballero 1997). Estimates of mutational variance are often based on the drift model of neutral mutations (Lynch and Hill 1986), in which genetic divergence among unselected lines from an isogenic base population is expected to increase linearly with time due to the accumulation of new mutations (but see Cockerham and Tachida 1987 for an alternative model). In most cases, the mutational variance is inferred by some form of least-squares regression in which an estimate is calculated from the slope of the regression of line divergence on the number of generations since separation of the lines from the base population (Lynch 1988a, 1994). Although there are problems with a standard least-squares approach (Lynch 1988a), using data from multiple generations simultaneously is desirable because of the high degree of uncertainty associated with estimating mutational variance based on measurements from a single generation (Lynch 1988a). Moreover, some long-term mutation accumulation studies show evidence for nonlinear divergence after many (>100) generations (Mackay *et al.* 1995). For these reasons, estimates of mutational variance based on repeated

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measurements from a single set of diverging lines are valuable.

With a few exceptions, investigations into the distributional properties of new mutations have ignored the age specificity of mutational effects. In many cases this is because the character of interest (*e.g.*, bristle number in Drosophila) is not expected to change with age in any biologically interesting manner. However, even when life-history characters are examined, age specificity is ignored in favor of a less informative, summary measure such as mean longevity or lifetime fecundity (Keightley and Caballero 1997). Direct experimental evidence for age-specific effects of mutations comes from only a handful of reports (Lynch 1985; Houle *et al.* 1994; Clark and Guadalupe 1995; Pletcher *et al.* 1998).

Pletcher *et al.* (1998), using *D. melanogaster*, reported larger estimates of mutational variance for mortality rates early in life (<28 days) when compared to older ages. Mutational effects on mortality rates were essentially uncorrelated between early and late ages. In contrast, higher mutational correlations (≈0.6) between fecundity at early and late ages in Drosophila were reported by Houle *et al.* (1994). Although additional experimental work is required before any general statements can be made about the frequency and effects of age-specific mutations, the data collected thus far have posed a challenge to some current ideas about life-history evolution (Pletcher and Curtsinger 1998; Promislow and Tatar 1998).

In this article, we present additional estimates of the age-specific properties of spontaneous mutations that affect mortality in *D. melanogaster*. These estimates are based on data collected after 10, 19, and 47 generations of mutation accumulation. The data from generation 19 were previously published (Pletcher *et al.* 1998), but those from generations 10 and 47 are included here for the first time. Novel maximum-likelihood methods (see Pletcher and Geyer 1999) were employed to obtain estimates of age-specific mutational variance and to examine the evolution of the genetic correlation between mortality rates at different ages through the course of the mutation accumulation. Although we find rather large fluctuations in genetic variance across generations—age-specific genetic variances in the generation 47 data were less than in the generation 19 data—we provide strong evidence that mutational variance is larger for early-life mortality rates than for mortality at older ages. Mutational correlations among mortality rates at different ages appear to increase with the accumulation of mutations.

MATERIALS AND METHODS

Stocks: In the Houle lab (University of Toronto), 100 mutation-accumulation (MA) lines were established from a single, inbred laboratory stock. These lines were independently maintained at very small population sizes (full-sib mating) to reduce the influence of natural selection on the allele frequency dynamics of nonneutral mutations. Subsets of the lines were assayed for mortality characteristics after 10, 19, and 47 generations of mutation accumulation. Details concerning the establishment and maintenance of the MA lines are provided in Pletcher *et al.* (1998).

Control populations were constructed through the use of cryopreservation. At the time of the initiation of the 100 MA lines, a large number of embryos from the base population were cryopreserved at Cornell University (see Houle *et al.* 1997). Several embryos were successfully revived at generation 10, but analysis of *P*-element insertion sites after the mortality experiment revealed cross-contamination among the control lines (S. Nuzhdin, personal communication). These lines were excluded from all analyses. Two males and three females were successfully revived at generation 19. Each female was used to found one of three replicate control populations. One male was mated with two of the females and the second was mated to the third female. Attempts to revive embryos at generation 47 were unsuccessful.

Control lines for the generation 47 experiment were derived from the generation 19 thaw. From the time of their establishment at generation 19, these control lines were each subdivided into two large populations, one of which was maintained at 17° and the other at 25°. Although the 17° populations may have had less opportunity to respond to selection, they may have evolved differently than lines maintained at 25°. Given the limited number of generations and the large effective population sizes, drift is not likely to be an important force in the dynamics of mutant allele frequencies. There are, however, ample opportunities for selection to act against deleterious mutations.

After 10, 19, and 47 generations of mutation accumulation, 12, 31, and 25 MA lines were randomly chosen for mortality analysis. Samples of flies from each line were used to generate two replicate populations (sublines), which were maintained independently and at large population sizes. This served two purposes: (i) to control for further mutation in the lines during their expansion to the large sizes required for mortality measurements and (ii) to allow an estimate of common environmental effects that might otherwise inflate estimates of the variation between lines generated by mutation.

Flies were sent from the Houle lab to the Curtsinger lab and immediately transferred into half-pint milk bottles with standard agar-yeast-molasses-cornmeal medium. The bottles were placed in a constant temperature (24°) and constant light walk-in incubator at ≈68% relative humidity. Flies from generations 10 and 19 were kept for 3 generations, during which time each line was expanded to six milk bottles to generate sufficient numbers of flies for the mortality measurements. Generation 47 flies required 4 generations and were expanded to eight milk bottles per line.

The ebony mutation in the experimental stocks provided an effective guard against undetected contamination from exogenous flies. To investigate the possibility of cross-contamination among marked lines, transposable element positions were analyzed (at generation 19) for the control lines and for 20 mutation-accumulation lines—10 with the highest fitness and 10 with the lowest (Pletcher *et al.* 1998). Two lines were identified as potentially contaminated. These lines were removed from all analyses.

Mortality measurements and estimation: A detailed description of the method for measuring and estimating mortality rates is given in Pletcher *et al.* (1998). In summary, age-specific mortality estimates were obtained using age-synchronized cohorts of flies. Each cohort was composed of ~800 flies (all emerging within a 30-hr window) from a specific

TABLE 1

Experimental designs for three mutation-accumulation experiments

	Generation		
	10	19	47
Experimental			
Lines	12	29 (10)	25 (3,17)
Flies	46,270	89,312	64,116
Control			
Lines	—	3	3
Flies	—	14,139	9,324

Summary of the number and type of experimental (mutation accumulation) and control lines used in each of three experiments. For generation 19, the value in parentheses indicates the number of lines that were included in both the generation 10 and 19 experiments. For generation 47, the parentheses indicate the number of lines included in the generation 47 analysis that were also included in the generation 10 and 19 experiments, respectively. Control lines were considered unique for each generation.

subline. Each chosen mutation-accumulation line was represented by four cohorts, two for each of two subline replicates. Cohorts were kept in plastic "population cages" in a constant light, temperature, and humidity walk-in incubator. Each day, every population cage was examined, and dead flies were removed, sexed, and recorded. Fresh medium was provided every other day, and cages were cleaned regularly (Pletcher *et al.* 1998).

The numbers of mutation-accumulation lines analyzed were 12, 29, and 25 for generations 10, 19, and 47, respectively. Some lines were measured in all three experiments, while others were only examined in one or two experiments. Each control population from each experiment was considered unique. Table 1 summarizes the size and line composition of the three experiments.

Age-specific mortality rates were estimated as $\hat{\mu}_x = -\ln(\hat{P}_x)$, where $\hat{P}_x = N_{x+1}/N_x$ is the probability of surviving from age x to age $x + 1$ given survival to at least age x (N_x is the number of individuals alive at the start of age x; Lee 1992).

Age-specific mutational variance: Although mortality rates were measured every day, they were pooled into weekly measures for three reasons: (i) to reduce the high degree of random fluctuation present in daily mortality rates; (ii) to decrease the number of ages that are analyzed, and (iii) to reduce the number of "zero death" age intervals. For short intervals, zero death ages are common very early in life (when mortality rates are low) and at the oldest ages (when sample sizes are small). They can bias variance component analyses (Pletcher *et al.* 1998; Promislow and Tatar 1998).

All variance component analyses were carried out on the natural logarithm of mortality, $\ln(\hat{\mu}_x)$. The maximum-likelihood techniques we use assume that the character of interest is normally distributed. The logarithmic transformation achieves this assumption as mortality rates are not significantly different from normal on this scale (experimentwise $P > 0.05$ using a Shapiro-Wilks test; data not presented).

Although each population cage is composed of many individuals, we have a *single* estimate of log mortality for each cage at each age. Estimates of the age-specific mutational variances and covariances were obtained using the "character process" approach outlined in a companion article (Pletcher and Geyer 1999). For comparison with earlier work (*e.g.*, Hughes and Charlesworth 1994; Promislow *et al.* 1996; Pletcher *et al.* 1998), estimates based on standard multivariate techniques are also presented.

TABLE 2

Covariance functions for within-generation analyses

Variance functions		Correlation functions	
Constant	θ_0	Normal	$\exp\{-\theta_c(s-t)^2\}$
Linear	$\theta_0 + \theta_1 t$	Cauchy	$\dfrac{1}{1+\theta_c(s-t)^2}$
Quadratic	$\theta_0 + \theta_1 t + \theta_2 t^2$	Uniform	$\dfrac{\sin\theta_c(s-t)}{\theta_c t}$

Functional forms for an arbitrary covariance function, $\rho(s, t) = V(s)V(t)r(s, t)$. $[V(T)]^2$ is termed the variance function, which describes the variance at age t, and $r(s, t)$ is the correlation function, which describes the correlation between character values measured at ages s and t. For all functions, the θ_i are estimated from the data using maximum likelihood under the restrictions that $[V(T)]^2 > 0$ for all t and $\theta_c \geq 0$.

The character process model: The character process model (Pletcher and Geyer 1999) is useful for the genetic covariance structure of characters that change as a function of some independent and continuous variable. In this specific case, log-mortality rate is the character and age is the continuous variable. By modeling log mortality as a time-dependent Gaussian stochastic process, we use a methodology that leads to an estimate of a *genetic covariance function*, $G(s, t)$, which describes the age-specific genetic variances of the character and the genetic covariance between the character expressed at ages s and t (see also Kirkpatrick and Heckman 1989). As with standard quantitative genetic analyses, covariance functions for other random effects (*e.g.*, between subline effects and between cage effects) are also estimated.

The general form for a covariance function is given by

$$\rho(s, t) = V(s)V(t)r(s, t), \tag{1}$$

where $V(s)$ is an arbitrary standard deviation function such that $[V(t)]^2$ is the variance at age t (*i.e.*, the variance function), and $r(s, t)$ is a positive-definite *correlation* function (Pletcher and Geyer 1999). Table 2 lists the different variance and correlation functions that were used in our analyses. See Pletcher and Geyer (1999) for a more complete discussion of the model.

The analysis is divided into two parts. First, in the within-generation analysis, data from each experiment are investigated separately using the standard character process approach (Pletcher and Geyer 1999). Second, the across-generation analysis uses data from all three experiments simultaneously to estimate the *expected* mutational variance for mortality at each age. In both cases, the between-line covariance function is of primary interest (it is directly related to the mutational variance), but variances among replicate sublines and among replicate population cages are estimated and accounted for as well.

Within-generation model: Treating the set of observed age-specific mortality rates from a single population cage as a realization of a stochastic process, we represent each observed process, $y(t)$, as being composed of four component processes,

$$y_{ijk}(t) = \mu(t) + l_i(t) + d_{j(i)}(t) + e_{k(ij)}(t), \tag{2}$$

where $\mu(t)$ describes the mean log mortality (over all MA lines) as a function of age, $l_i(t)$ is a process representing the

age-specific deviations from the mean function in MA line i, $d_{j(i)}(t)$ is a process representing the deviations due to sublines nested within each MA line, and $e_{k(ij)}(t)$ represents an environmental process that expresses the random environmental deviations. The $l(t)$, $d(t)$, and $e(t)$ processes are assumed to be uncorrelated and to have mean zero at each age. This is analogous to a random effects nested design for nonfunction-valued traits. In the case of single-valued traits we might estimate the among-line variance component. For function-valued traits we focus on the *covariance function* for the line effect, which describes variation in mortality due to genetic divergence among the lines. Covariance functions for the subline and random effects are also estimated.

For each experiment, a series of covariance functions were fit to the log-mortality data using maximum likelihood (Pletcher and Geyer 1999), and model comparisons were based on likelihood-ratio tests. For nested models, twice the difference in log likelihood is distributed as χ^2 with degrees of freedom equal to the number of additional parameters in the less-constrained model. Model comparison between nonnested models is more difficult (Cox 1961, 1962). In such cases, we compared the log likelihood of each model to the log-likelihood of a "supermodel" formed from a linear combination of the two separate models. Sexes were analyzed separately.

Estimates of mutational variance were obtained from the between-line covariance function assuming mutations were neutral, additive, and of small effect. For the character process model, the *mutational covariance function* is given by

$$\rho_m(s, t) = G(s, t)/2t, \tag{3}$$

where t is the number of generations of divergence and $G(s, t)$ is the covariance function of the $l(t)$ process in (2). This is a straightforward extension of the methods for single-valued characters (Lynch and Hill 1986; Lynch 1994).

Across-generation model: For these analyses, age periods were analyzed independently, and the continuous variable, t, represents time of divergence (rather than age as is the case in the within-generation analyses). Thus, for each fixed age period, x,

$$y^x_{ijk}(t) = \mu^x(t) + l^x_i(t) + d^x_{j(i)}(t) + e^x_{k(ij)}(t), \tag{4}$$

where $\mu(t)$ is the mean mortality rate (over all MA lines) in generation t, $l_i(t)$ is the mortality deviation at generation t for mutation-accumulation line i, $d_{j(i)}(t)$ represents the random deviation at generation t for the two genetic replicates nested within MA line i, and $e_{k(ij)}(t)$ is the error deviation at generation t.

As before, each random process in (4) has mean zero and an associated covariance function. On the basis of the infinitesimal model of mutation accumulation in the absence of selection (Lynch and Hill 1986), the variance function of the $l(t)$ process has the form

$$[V_l(t)]^2 = 4V_m + 2tV_m, \tag{5}$$

where V_m, the mutational variance, is a parameter to be estimated. The correlation structure is determined by the data. Various correlation functions were examined, and likelihood-ratio tests were used to determine the best model for the data.

To obtain a single estimate of environmental variance for each age, we took the mean value of the best-fit environmental variance function. Thus,

$$\hat{V}_e = \frac{1}{47}\int_0^{47} \hat{V}_E(t)^2 dt, \tag{6}$$

where $V_E(t)^2$ is the estimated variance function (see Equation 1) of the covariance function of the $e(t)$ process in (4). An identical procedure was used to estimate the between-replicate variance from the best-fit covariance function of the $d(t)$ process. Because each observed mortality rate is obtained from a group of individuals, our estimates of environmental variance are an underestimate of the actual environmental variation among individuals.

Standard multivariate model: To allow comparison of the generation 10 and 47 data with previously published results, estimates of age-specific variances obtained by treating each age as a separate character in a standard multivariate analysis were also calculated. Age-specific, between-line components of variance and covariance were estimated using QUERCUS (Shaw 1987; Shaw and Shaw 1992), and hypothesis testing was based on likelihood-ratio tests. Details of this procedure are provided in Pletcher *et al.* (1998).

Evolution of mean mortality rates: For the generation 19 and 47 experiments the average age-specific effects of mutations were examined by comparing the overall mean age-specific mortality rates of the MA lines to those from the controls. Means were calculated by averaging at each level of the design. Confidence intervals (95%) were calculated as twice the standard deviation of the line means at each age (N = 29 and 25 for the generation 19 and 47 MA lines, respectively; and N = 3 for the control lines at generations 19 and 47).

RESULTS

A very small percentage of population cages in each experiment exhibited high (>20 times the average) mortality rates beginning at eclosion and continuing throughout life. In large survival experiments, we typically observe such anomalies. These cages are considered outliers and are removed from the analysis. The fraction of excluded cages was 2/60, 2/142, and 1/115 for the generation 10, 19, and 47 experiments, respectively. A technical problem during the generation 19 experiment caused a short period of abnormally high male mortality early in life. This mortality was apparently randomly distributed among the cages, and no lasting effects were observed (Pletcher *et al.* 1998).

Within-generation analyses: Parameter estimates for the between-line covariance functions for each sex and each experiment are given in Table 3. In all cases, we chose the normal correlation function (see Table 2) to describe the genetic correlation between age periods. The normal usually provided the greatest likelihood when compared to the Cauchy and Uniform correlations functions (data not presented), but the significance of the increase in fit of the normal over the other correlation functions could not be determined. In all cases where two correlation functions were compared to a supermodel, the log likelihood of the supermodel was not significantly greater than either of the individual functions (data not presented). For all experiments, the best-fit environmental covariance function consisted of a linear variance function (with negative slope) and Cauchy correlation function. The best between-subline covariance function was either a constant or linear variance coupled with a normal correlation. For all experiments the magnitude of the between-replicate covari-

TABLE 3

Fitted covariance functions for mutation-accumulation lines after 10, 19, and 47 generations of accumulation

Sex	Generation	θ_0	θ_1	θ_2	θ_c
Females					
	10	0.04 (0.024)	—	—	30.3[a]
	19	0.43 (0.134)	−0.054 (0.023)	—	0.16 (0.053)
	47	0.13 (0.060)	−0.016 (0.009)	—	0.02 (0.022)
Males					
	10	0.04 (0.026)	—	—	0.19 (0.190)
	19	0.86 (0.197)	−0.318 (0.072)	0.03 (0.007)	0.32 (0.120)
	47	0.10 (0.030)	—	—	0.04 (0.024)

Within-generation estimates of the between-line covariance function. θ_0, θ_1, and θ_2 represent parameters of the variance function, while θ_c is a parameter for the correlation function (see Table 2 and Equation 1). In cases where values of θ_1 (θ_2) are provided, a linear (quadratic) variance function provided the best fit to the data. Otherwise, a constant variance across ages provided the best fit. Values in parentheses are the asymptotic standard errors of the estimates. In all cases, the normal covariance function (see Table 2) was fit to the data.
[a] The standard deviation could not be calculated.

ance was exceedingly small and not significantly different from zero (data not presented).

For females, there is strong evidence that the accumulation of spontaneous mutations generates significantly greater genetic variation for mortality rates early in life than at older ages (Table 3). Early in the mutation accumulation (generation 10), the best-fit variance function was a constant variance at all ages. Neither a linear nor quadratic function provided a significantly better fit ($P > 0.10$). Despite the limited number of generations of divergence, there is evidence for significant genetic variation among the MA lines (H_0: $\theta_0 > 0$; $P = 0.05$). For the generation 19 and 47 experiments, a linear variance function provided the best fit to the data (quadratic variance functions could not be estimated due to a lack of convergence). In both cases, the slope estimate θ_1 is negative, suggesting that genetic variation created by the accumulation of naturally occurring mutations is smaller at older ages. For example, in generation 19 the estimated between-line variance at age 1 wk is $\rho(1, 1) = 0.38$, but for 7 wk, $\rho(7, 7) = 0.052$. In generation 47 the absolute values of the variances are less, but the pattern is consistent. A significant ($P = 0.04$) linear term in the variance function describes a smaller genetic variance at older ages.

The genetic correlation between mortality rates at different ages appears to increase with the number of generations of divergence (Table 3). For the generation 10 data, the parameter of the normal correlation function was arbitrarily large, implying that mortality rates at adjacent age classes are genetically uncorrelated. However, because the standard deviation of this estimate is extremely large, there is no evidence that it is different from zero (*i.e.*, perfect correlation among all age classes). The correlation parameter estimate declines with increasing times of divergence. Although significantly greater than zero ($P = 0.0004$) at generation 19, we cannot distinguish it from zero at generation 47 ($P = 0.18$). Figure 1 illustrates the evolution of mutational correlation in females as a result of mutation accumulation.

The general pattern for males is similar to females. The best-fit variance function for the generation 10 and 47 experiments is a constant variance across ages. There is strong evidence, however, for a decline in variance with age in the generation 19 data ($P < 0.0001$). The quadratic variance function provides evidence for a slight increase in mutational variance at the oldest ages, but the increase is minimal. Age-specific genetic variances generated by mutation are smaller in males than in females in generations 19 and 47, but the correlations among ages are similar (Table 3). Although the genetic correlation between mortality rates at adjacent age peri-

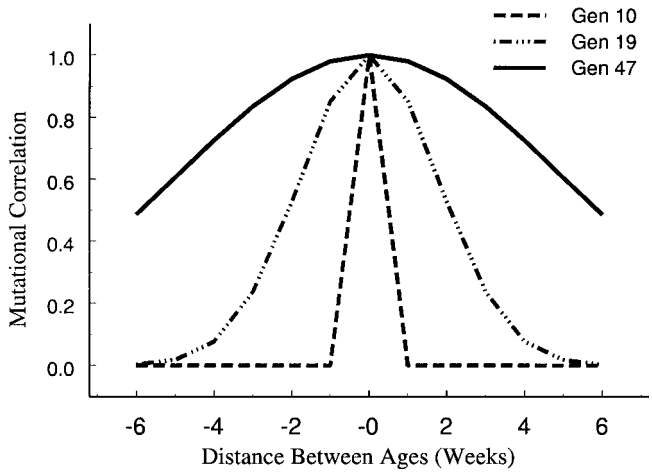

Figure 1.—Mutational correlations between weekly mortality rates in female Drosophila after 10, 19, and 47 generations of mutation accumulation. Estimates are based on the character process model (Pletcher and Geyer 1999) and are presented in terms of the time distance between age classes.

TABLE 4

Age-specific estimates of mutational variance based on 10, 19, and 47 generations of mutation accumulation

Sex	Age (wk)	V_m ($\times 10^{-3}$)	SE ($\times 10^{-3}$)	P	V_e	V_d
Females						
	1	5.15	1.58	0.0006	0.56	0.06
	2	5.96	1.65	0.0002	0.58	0.02
	3	4.05	1.32	0.001	0.65	0.06
	4	2.44	0.77	0.0008	0.44	0
	5	0.95	0.35	0.003	0.20	0
	6	0.04	0.14	0.391	0.22	0.05
	7	0.02	0.20	0.460	0.17	0
Males						
	1	1.11	1.00	0.134	0.85	0.10
	2	3.26	1.09	0.0014	0.52	0.001
	3	5.76	1.52	<0.0001	0.31	0.10
	4	2.17	0.57	<0.0001	0.19	0
	5	0.56	0.24	0.0094	0.11	0.02
	6	1.04	0.36	0.0018	0.17	0.004
	7	0.47	0.30	0.06	0.19	0

Estimates of the mutational variance, V_m, environmental variance, V_e, and the between-replicate variance, V_d, for mortality rates at different ages in Drosophila. Estimates are based on data from three separate experiments. The asymptotic standard errors of the V_m estimates and the P value for the null hypothesis that $V_m = 0$ are also given.

ods is slightly higher at generation 10 (θ_c in Table 3 is lower) than it is at generation 19, the generation 10 estimate has a relatively large standard deviation.

Across-generation analysis: Age-specific estimates of mutational variance obtained using model 4 and Equation 5 are given in Table 4. Point estimates of the between-replicate variation and the environmental variation were obtained from the best-fit variance functions of the respective processes and Equation 6. In all cases, the best-fit environmental variance function was linear in time with a negative slope. For ages in which the estimated between-replicate variance was nonzero, linear variance functions provided the best fit.

As suggested by the within-generation analyses (Table 3), mutational variance is higher earlier in life than it is at older ages (Table 4). For females, $V_m = 5.15 \times 10^{-3}$ for mortality in week 1, while $V_m = 0.04 \times 10^{-3}$ for mortality rates at week 6. After a Bonferroni correction for multiple hypothesis tests ($N = 7$; sexes separate), mutational variance is significantly greater than zero ($P \leq 0.007$) for weeks 1–5 in females and weeks 2–6 in males. The failure to detect significant mutational variance for week 1 mortality in males is likely due to the high random mortality at this age in the generation 19 data.

Standard multivariate model: Age-specific mutational variances estimated using standard multivariate techniques are presented in Table 5. The mutational variance at all ages is much lower for the generation 47 experiment than for the generation 19 experiment (Table 5). This is true for the environmental variances as well (data not shown). The age-specific estimates of V_m obtained from the character process model are within the range of values obtained from the three experiments individually. They are not a simple average of the indi-

TABLE 5

Estimates of age-specific mutational variance using standard multivariate techniques

Sex	Age (wk)	Gen. 10	Gen. 19	Gen. 47
Females	1	10.0	11.5***	1.6*
	2	3.0	11.5**	3.1**
	3	0.0	12.0**	1.6
	4	0.0	9.0***	0.4
	5	NC	1.3	0.9*
	6	NC	2.6*	0.04
	7	NC	0.0	0.05
Males	1	9.5	4.7	0.5
	2	3.0	7.9**	1.3
	3	0.25	13.9***	1.3
	4	0.0	3.9***	2.0**
	5	1.0	0.0	1.1**
	6	0.3	1.05	1.2*
	7	0.5	0.0	0.7*

Estimates of mutational variance, V_m, ($\times 10^{-3}$) for log-mortality rates for generations 10, 19, and 47 of mutation accumulation. Estimates were obtained by treating each age as a separate character in a standard multivariate analysis. Significance levels are provided for the null hypothesis that $V_m = 0$. ***$P <$ 0.0001; **$0.0001 \leq P \leq 0.007$; *$0.007 < P \leq 0.05$. NC, the maximum-likelihood algorithm failed to converge.

vidual estimates in part because the generation 19 and 47 experiments were much larger than the generation 10 experiment, and a different set of lines was examined in each experiment (see Table 1).

Evolution of mean mortality rates: Because attempts at reviving cryopreserved embryos were unsuccessful at generation 47 of the mutation accumulation, the lines recovered for the generation 19 experiment were used again at generation 47. Although the control populations were maintained at high population sizes to reduce the effects of drift, there was still the potential for deleterious mutations with small effects to influence age-specific mortality (Drake *et al.* 1998). If generation- or temperature-dependent effects were large, control lines maintained at 17° would be expected to show significantly different mortality rates than lines maintained at 25°. Mortality rates were not significantly different between the two types of control lines at any age (pointwise 95% confidence overlap at all ages; data not shown), and lines from the two temperatures were pooled for subsequent analyses. Although these results suggest that there was little change in the mortality rates of the control populations from generation 19 to generation 47, they should be interpreted with caution.

Average age-specific mortality rates for the mutation-accumulation lines and their corresponding control lines are given in Figure 2. Average rates were determined based on a total of three control lines for both the generation 19 experiment and the generation 47 experiment. For females, there is evidence that the mutation accumulation resulted in a net increase in mortality rates through middle ages (~10–25 days posteclosion) when compared to the corresponding control lines. Mortality rates very early in life and at older ages are nearly equivalent for both the mutation-accumulation and the control lines. In males, the accumulation of spontaneous mutations had little effect on mean mortality rates. For males of both the generation 19 and 47 experiments, the average mortality rates from the MA lines are nearly identical to those from the respective control lines (Figure 2).

DISCUSSION

Significant levels of genetic variation caused by the accumulation of naturally occurring spontaneous mutations were observed for age-specific mortality rates in Drosophila. Mutational variances were obtained using new survival data from 25 mutation-accumulation lines allowed to accumulate mutations for 47 generations. These data were combined with data from generations 19 (previously published) and 10 (previously unpublished) to estimate mutational properties. Both within- and among-generation analyses suggest that the input of genetic variance by mutation is greater early in life than at older ages, and no significant mutational variance was detected at the oldest ages (6- and 7-wk posteclosion in females and 7-wk posteclosion in males). Mutational correlations among mortality rates at different ages appear to increase with the accumulation of mutations. There is little evidence for mutation bias with respect to age-specific mortality.

Age-specific properties of mutations: Analyses that examine data from each of the three experiments individually (*i.e.*, the *within-generation* analyses) suggest that mutational variance is lower at older ages (Table 3) than it is early in life. This is evidenced by a statistically significant negative slope parameter in the best-fit variance functions at generations 19 and 47 for females and generation 19 for males. Previous estimates of age-specific genetic and mutational variation in Drosophila suggest a quadratic relationship between age and genetic variance with a peak near 3 wk of age (Promislow *et al.* 1996; Pletcher *et al.* 1998). Our data do not support such a trend. The quadratic variance function was only accepted for the male, generation 19 data, and it describes a steady decline in variance until very old ages after which there is a slight increase.

The decrease in the correlation function parameter over the course of the mutation accumulation suggests an increase in the genetic correlations among mortality rates (Table 3 and Figure 1). However, only three periods of divergence are examined and the correlation estimates all lie within two standard deviations of each other. It is difficult to say whether or not this trend is real.

Comparison of the levels of between-line variance in the three experiments reveals that the overall levels of variation are much smaller in the generation 47 experiment than in the generation 19 experiment (Tables 3 and 5). This is disturbing, considering that we expect the between-line variance to increase linearly with the number of generations of divergence. There are several possible explanations for this observation. First, there may be considerable "block" effects. These experiments were carried out at different times, nearly a year apart, and mortality measurements are known to be sensitive to environmental conditions (Curtsinger *et al.* 1992; Hughes and Charlesworth 1994). One considerable source of block effects may be the first author's increasing experience with mortality measurements. Age-specific phenotypic variances decrease with generation number (data not shown), and this no doubt reflects better experimental procedures employed in the later experiments. Another manifestation of such effects might take the form of scale-dependent differences in the variances resulting from large differences in mean mortality across generations. However, mortality rates in the generation 47 data are, for the most part, not significantly different from the generation 19 data (Figure 2). In general, mortality is slightly higher at all ages in the generation 47 experiment, making this explanation implausible.

It is also likely that there are actual fluctuations in

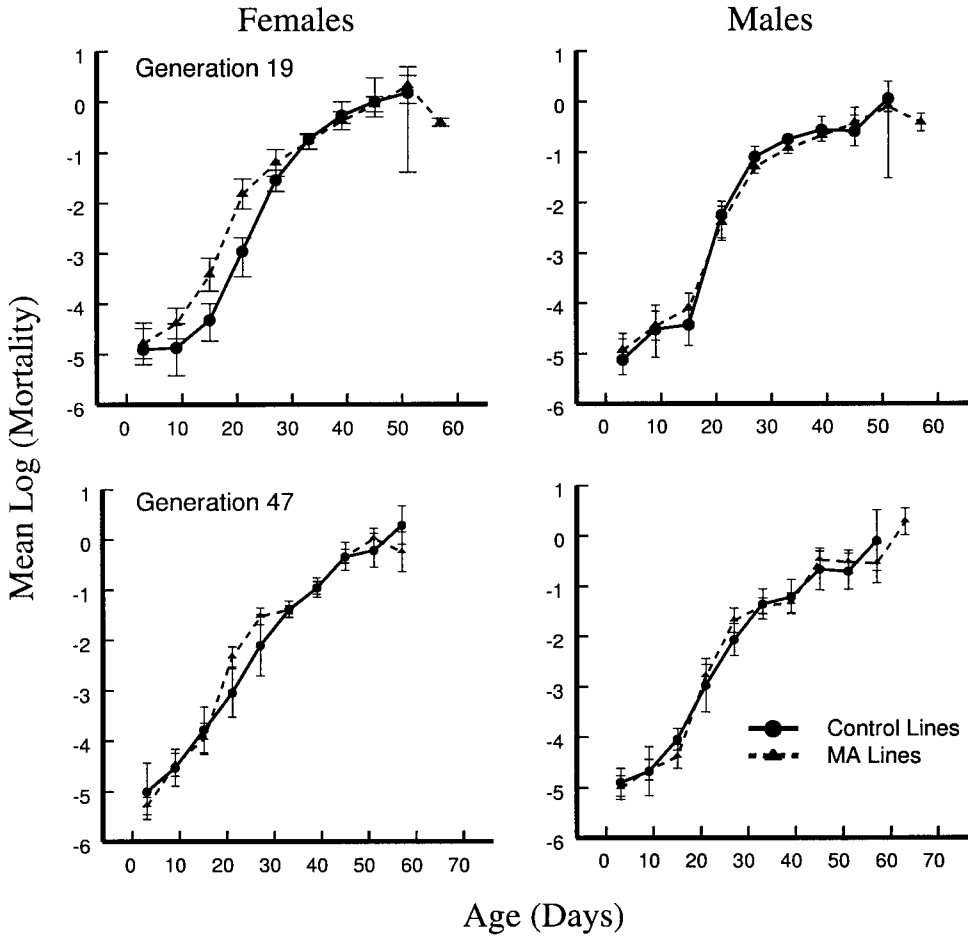

Figure 2.—Mean mortality rates (and pointwise 95% confidence intervals) for male and female Drosophila. Dashed lines represent mutation-accumulation lines after 19 and 47 generations of mutation accumulation (N = 29 for generation 19; N = 25 for generation 47), and solid lines represent control lines (N = 3 for generations 19 and 47).

the among-line variance around its expectation. When the number of lines examined is small (<100), these fluctuations can be substantial (Lynch 1988a). Moreover, previous mutation-accumulation experiments that measured genetic variance often and over a long period of time reveal dramatic, short-term variation in estimates of mutational variance (Mackay *et al.* 1994, 1995). Despite such variation, among-line variation tends to increase linearly with the number of generations of divergence. There is some suggestion of a leveling off of the among-line variance for bristle number in Drosophila (Mackay *et al.* 1995), but this was not observed until well after 100 generations of divergence.

If block effects and variation in the estimates of among-line divergence are taken into consideration, the use of data from all three experiments provides the best estimate of the *expected* increase in among-line variance per generation and therefore the best estimate of mutational variance. Although the infinitesimal model of Lynch and Hill (1986) formed the basis for our estimates of V_m, house-of-cards models (Cockerham and Tachida 1987) are nearly identical for the periods of divergence in this study (Cockerham 1994). These *across-generation* analyses support the conclusions drawn from the within-generation models. Mutational variances at early ages are significantly greater than at older ages. An order of magnitude separates the largest and smallest age-specific values in each sex (Table 4).

Early in the mutation accumulation we find significant age-specific mutational variance and mutational correlations that rapidly decline as ages become further separated in time. This suggests a prevalence of age-specific mutation effects (Pletcher *et al.* 1998). In later generations the mutational correlation is much higher between adjacent age classes and between increasingly disparate ages. Contrary to our suggestion after 19 generations of accumulation (Pletcher *et al.* 1998), these results are consistent with those reported by Houle *et al.* (1994), who report a mutational correlation of ≈0.6 for early- and late-life fecundity after 44 generations of mutation accumulation.

The buildup of genetic correlations is perplexing, and to our knowledge there are no life-history models that predict this observation. We can hypothesize two situations that might generate such a pattern. One is synergistic epistasis among age-specific mutational effects. If a mutation that affects one age class increases the likelihood or effects of mutations affecting another age class, the correlation among age classes would increase with time as certain lines become increasingly "sick." We found no evidence for among-line correlations across generations that would suggest that lines

with relatively high mortality at, say, generation 19 also had high mortality at generation 10 or 47. Unfortunately, the number of lines examined in multiple generations is small, and it is likely we have little statistical power to detect such a trend. Second, there may be two classes of mutations: one with small, age-specific effects and the other with rather large effects throughout life. Evidence for both types of mutations is reported in Pletcher *et al.* (1998). If the rate of age-specific mutation is higher, mutational variance would be observed rather early, while high correlations across ages would not be observed until late in the accumulation. This explanation requires an extremely low mutation rate to pleiotropic alleles such that none would be expected to occur for many generations. Controlled mutagenesis experiments that measure the effects of a known number of mutations on age-specific mortality are needed to distinguish these possibilities.

The data provide no evidence for negative mutational correlations between mortality rates at any age. Although the normal correlation function restricts the correlation between age classes to be greater than zero (Table 2), the uniform correlation function can be negative in sign. When this function was fit to the data, the estimated correlations were strictly positive within the range of our data for generations 10 and 47. The correlation function did become negative between weeks 1 and 7 for both sexes in the generation 20 data, but the value of the correlation was not significantly different from zero ($P = 0.44$ and $P = 0.49$ for females and males, respectively).

Evolution of mean mortality rates: With the exception of an increase in mortality rates from 15–30 days in the generation 19 females, there is no evidence for a mortality bias in the effects of new mutations (Figure 2). Except for this slight "bump," mortality rates in mutation-accumulation and control lines are nearly identical. These small changes in mean mortality rates are consistent with recent observations in fitness characters of *Caenorhabditis elegans* (Keightley and Caballero 1997). They contrast with the 1–2% reductions in mean viability per generation reported in Mukai (1964) and Mukai *et al.* (1972).

One partial explanation for the lack of mutational bias derives from the culture conditions experienced in the laboratory by both the control and mutation-accumulation lines. Both sets of lines were maintained on 2-wk generation cycles during the experiment. Moreover, the inbred line used to found both sets of lines was maintained on this schedule for many (>50) generations. Mortality rates after week 1 are essentially neutral in this culture regime (Promislow and Tatar 1998; D. Houle and L. Rowe, unpublished data), suggesting the possibility that late-life mortality may have already been increased by mutation to maximal levels.

The lack of bias very early in life remains unexplained under this hypothesis. It may be that with pleiotropy involving enough fitness components such as larval viability, fecundity at various ages, development time, etc., a new mutation might raise some components of fitness even while depressing fitness overall. This is, however, somewhat contradictory to published data (Houle *et al.* 1994), which indicate positive correlations between fitness components.

Nonevolutionary influences on age-specific variance: There are several factors other than mutational effects that may have influenced our results (Promislow *et al.* 1996; Pletcher *et al.* 1998). Most importantly, nongenetic and developmentally acquired variation among individual flies can lead to biases in variance component estimates (Promislow and Tatar 1998; S. Pletcher and J. Curtsinger, unpublished data). Frail individuals tend to die sooner, causing population level mortality rates to drop. In principle, this could influence variation in log-mortality rates between lines. A quantification of this effect has not been worked out, but theoretical work investigating its significance is currently under way (S. D. Pletcher and J. W. Curtsinger, unpublished data).

The reduced sample size at older ages might result in a significant reduction of statistical power to detect mutational variance. This issue was addressed in Pletcher *et al.* (1998), using computer simulations. A significant reduction of statistical power was not observed until week 7. A substantial bias toward underestimating the true degree of genetic variance was seen in week 6, but this is not sufficient to generate the range of age-specific mutational variances seen in our data.

As with many mutation-accumulation studies, we did not possess ideal control lines. Although at this time there is no detailed information concerning the genetic effects of cryopreservation in Drosophila, Houle *et al.* (1997) report no increase in the rate of lethal mutations as a result of this process. There remains, however, the possibility that freezing introduces relatively small, consistent changes in the genome. The failure of the generation 47 thaw required us to use the generation 19 control lines. Although maintained at large population sizes, there is no guarantee that unmeasured genetic changes through selection or drift did not influence our results.

The evolution of senescence: The idea that new mutations may have age-specific effects and that the distribution of these effects is dependent on the age of expression dates back to Medawar (1952) and his argument for the evolution of senescence. Medawar suggested that senescence arises from the accumulation of late-acting, deleterious mutations, which can persist in a population due to the decline in the force of selection with advancing age. This idea has come to be known as the mutation-accumulation theory of senescence. A second model of senescence, antagonistic pleiotropy, assumes the existence of mutations with beneficial effects at early ages and deleterious effects later in life (Williams 1957). Again, because the force of selection is weaker at older

ages, these pleiotropic mutations are favored by selection.

Although these early assumptions about mutational effects served to frame arguments about senescence, models based solely on age-specific and/or pleiotropic mutations enjoy only modest support from the available experimental data (Curtsinger *et al.* 1995; Pletcher and Curtsinger 1998). Charlesworth (1990) showed that a model of senescence based only on mutations with simple, age-specific effects predicts a monotonic increase in genetic variance with age and a wall of postreproductive mortality near 100%. Contrary to predictions, Shaw *et al.* (1999), reanalyzing data from Promislow *et al.* (1996) and Hughes and Charlesworth (1994), found evidence for an initial increase and then decrease in genetic variance with age. Further, unless arbitrary assumptions are made about the number of loci affecting mortality rates at each age, the existence of mortality plateaus well below a 100% probability of death at the oldest ages (Vaupel *et al.* 1998) is contrary to both mutation-accumulation and antagonistic pleiotropy models (Pletcher and Curtsinger 1998).

Before a tenable theory for the evolution of age-specific mortality rates can be developed, more information on the age-specific properties of new mutations is required (Pletcher and Curtsinger 1998; Promislow and Tatar 1998). Our data suggest there may be different classes of mutations occurring simultaneously, and it may be that models based on mixtures of mutations may be consistent with the available demographic observations. Rather arbitrary patterns of age-specific genetic variance might be caused by relatively common, age-specific mutations that permit subtle evolutionary changes in the shape of the mortality curve. Rare, age-independent mutations might generate positive correlations among age-classes over time, and this "positive pleiotropy" might be important in maintaining low levels of mortality well into advanced ages.

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