Properties of Ethylmethane Sulfonate-Induced Mutations Affecting Life-History Traits in *Caenorhabditis elegans* and Inferences About Bivariate Distributions of Mutation Effects

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ABSTRACT

The homozygous effects of ethylmethane sulfonate (EMS)-induced mutations in *Caenorhabditis elegans* are compared across life-history traits. Mutagenesis has a greater effect on early than late reproductive output, since EMS-induced mutations tend to cause delayed reproduction. Mutagenesis changes the mean and variance of longevity much less than reproductive output traits. Mutations that increase total or early productivity are not detected, but the net effect of mutations is to increase and decrease late productivity to approximately equal extents. Although most mutations decrease longevity, a mutant line with increased longevity was found. A flattening of mortality curves with age is noted, particularly in EMS lines. We infer that less than one-tenth of mutations that have fitness effects in natural conditions are detected in the laboratory, and such mutations have moderately large effects (~20% of the mean). Mutational correlations for life-history traits are strong and positive. Correlations between early or late productivity and longevity are of similar magnitude. We develop a maximum-likelihood procedure to infer bivariate distributions of mutation effects. We show that strong mutation-induced genetic correlations do not necessarily imply strong directional correlations between mutational effects, since correlation is also generated by lines carrying different numbers of mutations.

MUTATIONS provide the source of all genetic variation among individuals and the basis for evolutionary change. Yet, relatively little is known about the distribution of effects and properties of new mutations for fitness-related traits. One method of studying the fitness effects of new mutations involves the accumulation of spontaneous mutations in inbred sublines under conditions of minimal selection, followed by measurement of the distribution of life-history traits in these mutation-accumulation lines and controls. Such experiments, the largest body of work by Mukai and associates in the 1960s and 1970s (Mukai 1964; Mukai *et al.* 1972; Ohnishi 1977), have recently proliferated in number (for recent reviews, see Charlesworth and Charlesworth 1998; Garcia-Dorado *et al.* 1999; Keightley and Eyre-Walker 1999; Lynch *et al.* 1999), partly due to a renewed interest in genome-wide mutation rates and the potential implications of recurrent spontaneous mutations for the evolution of sex and genetic load.

Fitness, however, is a complex trait. Inferring the effects of mutation on life-history traits is clearly valuable, but only gives a part of the picture. The total number of mutations affecting overall fitness may be underestimated if only one component of fitness is measured. Furthermore, if a single mutation has pleiotropic effects on two or more traits, the overall effect of that mutation on fitness may be underestimated if only one of the traits is measured. Thus, for a fuller picture of the effects of mutations on fitness, multiple life-history traits and the correlations between them should be measured. Correlated effects of mutations are of interest in a broader sense as well: genetic correlations are important in the context of correlated responses to selection and may act as constraints on evolutionary change (Falconer and Mackay 1996).

Estimates of mutational correlations have previously been obtained from two mutation-accumulation experiments, both in Drosophila. Houle *et al.* (1994) measured a series of life-history traits in chromosome balancer-derived lines, including competitive fitness, total, early, and late productivity, and longevity, and reported strong positive genetic correlations in all cases. In contrast, Fernandez and Lopez-Fanjul (1996) reported low values (maximum 0.25) for genetic correlations between viability traits and fecundity.

Mutational effects on multiple traits and some mutational correlations have particular relevance to specific evolutionary models. In particular, the effects of mutations on early and late reproduction are important in the context of models of senescence. Senescence, the deterioration of fertility and fitness with age, occurs almost universally. This presents a problem for evolu-

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tionary biologists: if organisms can function well in youth, why should they not continue to do so? An answer to this is provided by evolutionary theories of aging, which state that natural selection will tend to put a greater relative weight on mutations that affect survival and other components of fitness that act early in life than those that affect later stages, because, by the time alleles with influences later in life take effect, more of the original carriers will have died or become infertile for other reasons (Medawar 1946, 1952; Williams 1957; Hamilton 1966). This process will lead to the evolution of a life history in which fertility and survival chances decrease with increasing age.

Theoretical work has identified two possible paths by which this age-specific selection pressure can lead to the evolution of aging. The first suggests that it may be caused by the accumulation of mutations that have deleterious effects on fitness late in life (the mutation-accumulation model; Edney and Gill 1968; Partridge and Barton 1993; Charlesworth 1994). The second states that those alleles with beneficial effects early in life, but that can have harmful effects later, will be favored over those that produce beneficial effects later, and so the optimal life history will include decline in fitness later in life (the optimality or antagonistic pleiotropy model; Williams 1966; Rose 1982; Partridge and Barton 1993). This model assumes a negative correlation between early and late fitness components, such as early productivity and lifespan. To date, experimental studies of the relationship between fitness components and longevity have focused on standing variation in populations (Hughes and Charlesworth 1994; Promislow *et al.* 1996), differences between selection lines (reviewed by Zwaan 1999), specific mutants of large effect (reviewed by Kenyon 1997), and quantitative trait loci (Shook *et al.* 1996; Nuzhdin *et al.* 1997). As noted by Charlesworth (1993), "further information on the properties of mutational variation is badly needed."

Previously (Davies *et al.* 1999), we reported an EMS mutagenesis experiment in *Caenorhabditis elegans* in which we studied the effects of induced mutations on reproductive output. The mutagenesis was carried out using a standard dosage (50 mm EMS for 4 hr), for which the number of point mutations (predominantly G/C to A/T transitions) induced in the DNA has been calibrated (Anderson 1995; Davies *et al.* 1999). The lines were then inbred for 10 generations in conditions designed to minimize selection, to fix the new mutations, and life-history assays were carried out. Here, we extend the analysis of the EMS lines to a suite of life-history traits: various measures of productivity (total, early, and late), lifespan, and "relative fitness," a fitness measure appropriate for an age-structured population at equilibrium. We compare the effects of EMS treatment on these traits in terms of changes of mean and variance and estimated numbers of mutations induced. We focus on the joint distributions of mutational effects on the traits measured, in particular the joint effects of mutations on longevity and the other major fitness components. We develop a maximum-likelihood procedure to estimate properties of the bivariate distribution of mutation effects. One advantage of using *C. elegans* as a model system is the ease with which a highly inbred, genetically homogeneous population may be obtained and studied. Because the lines are highly inbred, there is expected to be no genetic variance between individuals within a line, and thus the residual component of variance must be purely environmental. By inducing large numbers of mutations, we can increase the likelihood of finding mutations with pleiotropic effects, although distinguishing between individual mutations with effects on more than one trait and associations between mutations that affect different traits becomes more challenging.

MATERIALS AND METHODS

Mutagenesis and generation of *C. elegans* lines: The EMS mutagenesis procedure and the derivation of the *C. elegans* EMS and control lines used in this study have been described in detail elsewhere (Davies *et al.* 1999). Briefly, the wild-type strain N2 was mutagenized with 50 mm EMS for 4 hr at 20° according to the protocol of Anderson (1995). This is expected to generate ~220 G/C → A/T transition mutations per haploid genome, ~50 of which cause amino acid mutations in protein coding genes (Anderson 1995; Davies *et al.* 1999). From the mutagenized worms, 60 lines were inbred under minimal selection by the transfer of single hermaphrodite larvae, chosen at random each generation, to randomly fix induced mutations. Unmutagenized control lines (40) were treated in an identical manner. Backup cultures were employed for cases in which a worm failed to reproduce. By the 10th transfer, there were 56 surviving EMS lines and 40 control lines.

Life-history trait assays: Daily reproductive output and lifespan of individual worms were recorded. Individual replicates of each line were maintained for three generations prior to each assay to remove the influence of maternal effects. Traits were measured contemporaneously for all EMS and control lines. Each of three people assayed one worm from each line, and the entire assay was repeated three times, giving a total of nine worms assayed per line. The numbers of progeny surviving to the L3 stage that were produced by individual worms during the first 6 days of their reproductive period were counted (reproduction starts on day 4). A combined progeny count was carried out for the last 2 days of reproduction, since almost all progeny are produced in the first 5 days of the reproductive period. The day on which the parental worm died was recorded. A worm was scored as dead if it ceased to respond to light touch with a platinum pick and showed a loss of turgor, or showed visible signs of decay. We concentrated our analysis on five traits: total productivity, early productivity (offspring produced during the first 2 days of the reproductive period), late productivity (offspring produced during the remaining 4 days), longevity, and relative fitness, a measure related to intrinsic population growth rate (Charlesworth 1994). To calculate values for relative fitness, the intrinsic growth rate, r_i, of each control line was computed by solving

$$\sum_x e^{-r_i x} l_i(x)\, m_i(x) = 1 \qquad (1)$$

using Newton-Raphson iteration, where $l_i(x)$ and $m_i(x)$ are proportions of worms surviving to day x and fecundities at day x, respectively, for line i. The relative fitness, w_{ij}, of each EMS or control individual was computed from

$$w_{ij} = \sum_x e^{-r_c x} l_{ij}(x)\, m_{ij}(x) \qquad (2)$$

(Charlesworth 1994, p. 120), where r_c is the average intrinsic growth rate for the control lines, and $l_{ij}(x)$ and $m_{ij}(x)$ are survival probabilities and fecundities, respectively, to day x for worm j of line i. Evaluation of (2) gave a mean relative fitness for the control lines very close to 1. The above fitness measure is appropriate for a population whose age structure is at equilibrium (Charlesworth 1994) and is preferable to using r itself (Vassilieva and Lynch 1999; Keightley and Bataillon 2000).

Comparison of effects of mutagenesis: An aim of the experiment was to compare the effects of EMS mutagenesis on a range of life-history traits. We employed three measures of "mutational target" size to make such comparisons. One measure is the scaled change in mean trait value, M, between the EMS and control (CON) lines, $\Delta M/M = (M_{\mathrm{CON}} - M_{\mathrm{EMS}})/M_{\mathrm{CON}}$. Two other measures are based on the EMS-induced genetic variance, V_{G}, which was obtained as the difference between genetic variance components of the EMS and control lines, from analysis of variance (ANOVA). The two measures based on variance are the mutational "heritability," $h^2_{\mathrm{M}} = V_{\mathrm{M}}/V_{\mathrm{E}}$, where $V_{\mathrm{M}} = V_{\mathrm{G}}/2$ and V_{E} is the environmental variance of the controls, and the mutational coefficient of variation, $\mathrm{CV_M} = V_{\mathrm{M}}^{1/2}/M_{\mathrm{CON}}$. Variances attributable to the factors in the experiment were inferred from ANOVAs, in which effects were fitted for measurer and assay number and their interaction, and, where significant, line–measurer–assay number interaction. Standard errors for h^2_{M} and $\mathrm{CV_M}$ were obtained by bootstrapping the data by line, 100 times.

Univariate estimation of mutation numbers and effects: We estimated mutational parameters using the Bateman-Mukai (BM) approach (Bateman 1959; Mukai 1964; see also Lynch and Walsh 1998) and by maximum likelihood (ML; see Keightley and Ohnishi 1998 for details). The BM approach assumes equal mutation effects and uses the change of mean between the treated and untreated lines and the genetic variance of the treated lines to estimate an "effective" number of mutations per haploid, U_{I}, along with a mutational effect parameter, s. Under ML, the same equal-effects model as under BM can be assumed, or mutation effects can be assumed to come from a chosen family of distributions. The fit of different distributions to the data is compared via the likelihood. Under the gamma distribution model assumed here, the distribution parameters estimated are shape β, and mean mutational effect $\bar{s} = \beta/\alpha$, where α is a scale parameter. To keep the computations manageable, we assume unidirectional (*i.e.*, unreflected) gamma distributions. The likelihood calculations used line means, whose distributions for the controls are close to normal. Estimates based on line means were very similar to those based on individual worms.

Estimation of genetic correlations between life-history traits: Estimates of genetic and environmental correlation coefficients between the life-history traits were obtained by restricted maximum likelihood using the average information algorithm implemented in the ASREML package (Gilmour *et al.* 1995). This analysis fully accounts for the slight imbalance that was present in the data. Effects were fitted for line, measurer, and replicate, plus measurer-by-replicate interaction. The genetic (co)variance is computed as the difference between EMS and control line genetic (co)variance estimates, and the genetic correlation, r_{G}, computed directly from these. The environmental correlation is computed from the control line data. Standard errors for correlation coefficient estimates were obtained by carrying out the above analysis on bootstrapped data (by line) 100 times.

Likelihood approach to infer bivariate mutation distributions: We assumed that mutations had unidirectional effects on traits X and Y, that all mutations had some effect on both traits, and, for the purposes of the analysis described below, that the correlation between mutational effects was positive. The number of mutations fixed per line was assumed to be a random variable from a Poisson distribution with parameter U_{I}, and the mutation effects were assumed to follow a bivariate gamma distribution with correlation between mutational effects ρ, scale parameters α_X and α_Y, and the same shape parameter, β, for each trait. The same β is not a requirement, but was assumed to reduce the computational complexity and to reduce the dimensionality of the parameter space to be searched. The environmental deviates were assumed to follow a bivariate normal distribution with variances V_{E}^X and V_{E}^Y and covariance $\mathrm{cov_E}$. To speed up the computations, the likelihood calculations were set up as appropriate for line mean values. Under the above assumptions, the likelihood associated with line i with phenotypic values Z_i^X, Z_i^Y is the bivariate analog of the likelihood Equation 2 of Keightley and Ohnishi (1998),

$$\begin{aligned} L(Z_i^X, Z_i^Y | \alpha_X, \alpha_Y, \beta, \rho, U_{\mathrm{I}}, M_X, M_Y, V_{\mathrm{E}}^X, V_{\mathrm{E}}^Y, \mathrm{cov_E}) \\ = p(0|U_{\mathrm{I}})\, f(Z_i^X, Z_i^Y | M_X, M_Y, V_{\mathrm{E}}^X, V_{\mathrm{E}}^Y, \mathrm{cov_E}) \\ + p(1|U_{\mathrm{I}}) \iint f(Z_i^X + a, Z_i^Y + b | M_X, M_Y, V_{\mathrm{E}}^X, V_{\mathrm{E}}^Y, \mathrm{cov_E}) \\ \times h(a, b | \alpha_X, \alpha_Y, \beta, \rho)\, da\, db \\ + p(2|U_{\mathrm{I}}) \iint f(Z_i^X + a, Z_i^Y + b | M_X, M_Y, V_{\mathrm{E}}^X, V_{\mathrm{E}}^Y, \mathrm{cov_E}) \\ \times h(a, b | \alpha_X, \alpha_Y, 2\beta, \rho)\, da\, db + \ldots, \end{aligned} \qquad (3)$$

where $p(x|U_{\mathrm{I}})$ is the Poisson distribution function for x mutation events, $f()$ is the bivariate normal density function, and $h()$ is the bivariate gamma distribution function. Equation 3 makes use of the fact that the sum of n pairs of gamma deviates with parameters ρ, α_X, α_Y, β is also bivariate gamma distributed with parameters ρ, α_X, α_Y, $n\beta$. The overall likelihood of the data was the product of likelihoods for each line, and control line data were included with U_{I} set to zero.

Equation 3 was evaluated numerically in a way similar to that described by Keightley and Ohnishi (1998). The double integrals were evaluated using precomputed tables of bivariate gamma frequency distributions of mean 1 and a large range of β values. These tables were generated by the "GTVR" algorithm of Schmeiser and Lal (1982). The β values were multiples of 0.25, allowing evaluation of the overall likelihood for β values that are a multiple of 0.25. Sets of tables were generated for 11 values of ρ: 0, 0.1, 0.2, . . . 1. Each table was subdivided into subranges to improve the spread of the distribution of frequencies and hence increase the accuracy. Tables of dimension 20×20 were used in an initial grid search (see below), and then 50×50 tables were used in a "final" likelihood maximization. The precision obtained for tables of different dimension was compared by analysis of simulated data sets; it was found that 100×100 tables gave the same likelihood to the first decimal place as 50×50 tables and profile likelihoods that were indistinguishable. The 20×20 tables were used to speed up the initial grid searches. These tables also gave similar-shaped profile likelihoods, but likelihood values differed in the first decimal place.

Likelihood maximization: A combination of grid searches and the simplex method (Nelder and Mead 1965) was used to maximize likelihood. Likelihood was separately maximized for the 11 fixed values of ρ (0, 0.1, etc.) and a series of fixed values of β (note that β and ρ were not varied within likelihood maximizations). Likelihood was maximized with respect to

the remaining parameters using the simplex algorithm, but to reduce the dimensionality, initial searches with five fixed values of U_I, varying by a factor of four, were carried out. To verify the ML procedure, we analyzed sets of simulated data that conformed to the analysis model assumed. In these analyses, the middle value of U_I in the initial search was the simulated value. In the analysis of *C. elegans* life-history trait data, the middle value was similar to the univariate estimate of U_I for one of the two traits. Starting values for the remaining parameters were computed from the data. Starting values for α_X and α_Y were functions of U_I, β, and the change of mean, ΔM, between the control and mutated lines, *e.g.*, $\alpha_X = U\beta/\Delta M_X$. Starting values for M_X, M_Y, V_E^X, V_E^Y, and cov_E were computed from means and (co)variances of the control line means. The final simplex (using the 50×50 bivariate gamma tables) involved maximization for U_I, α_X, α_Y, M_X, M_Y, V_E^X, V_E^Y, and cov_E and used starting values that had given the highest likelihood in the initial search over U_I values. After each maximization had converged, including the initial searches, the simplex was restarted from the point of initial convergence to check that convergence was genuine, as recommended by Press *et al.* (1992).

RESULTS

EMS-induced variation for life-history traits: A summary of results from ANOVA of control and EMS line data is shown in Table 1. Variation among control lines is nonsignificant for all traits, but it is highly significant among EMS lines in all cases. There are significant assay number effects for each trait and both treatments, reflecting unexplained environmental differences between the three assays; such effects on life-history traits have previously been noted in *C. elegans* (Johnson and Hutchinson 1993). For this reason, each measurer assayed each EMS and control line in each assay. Measurer effects and assay–measurer interactions are significant in several cases, probably reflecting changing relative levels of experience in carrying out worm assays among the three measurers. For two traits, early productivity and relative fitness, there are significant assay–line and measurer–line interactions, implying that specific lines reacted differently to different environments (assays), and that specific lines were treated or measured differently by different measurers, although this must have been inadvertent since plates were randomized.

Mutational target sizes—changes of means and variances: The effects of EMS mutagenesis on the means and variances for life-history traits are compared in Table 2, and the distributions of line means are shown in Figure 1. Comparison of the scaled change in mean, $\Delta M/M$, shows that the EMS mutagenesis had the greatest effects on early (days 1–2 of reproduction) productivity and relative fitness. As shown later, the genetic correlation of these traits is close to 1. Reduced early reproduction seems to be brought about partly by delayed reproduction, presumably due to an increase in mean development time, and this also resulted in an increase in mean late (days 3–6 of reproduction) reproductive output. The effect of EMS in delaying reproduction can be seen in more detail in Figure 2.

In terms of mutational heritability, however, total productivity is a larger target than early productivity, presumably reflecting a higher environmental variance for early productivity. Longevity is a substantially smaller mutational target that any other trait measured by any of the criteria. Late productivity seems to be influenced more or less equally by mutations that increase or decrease the trait (Figure 1); increases presumably are the result of delayed development. In the case of longevity, the distribution of EMS line means is skewed downward, but there is also an indication that one or two lines have higher longevity than the controls. One EMS line has a mean longevity of 16.0 days, which is 2.6 phenotypic standard deviations ($\sim$25%) above the control mean. The increased life span of this line was found to be replicable (C. Greer, unpublished data). Of the remaining 55 lines, 5 had longevity significantly lower than the control mean, but none had significantly increased longevity (after Bonferroni correction). For traits other than longevity and late productivity, there is no evidence in this experiment of mutations that increase the trait value.

Mutational target sizes—mutation rates and effects: Changes of means and variances (Table 2) depend jointly on the numbers of mutations induced and their distributions of effects. We investigated the underlying mutational parameters by applying the BM method of moments and ML to all traits except late productivity. In both analyses, we assumed that mutations unconditionally reduce the trait value. Under the BM analysis, the results suggest that each line is affected by only a few mutations, or in the case of longevity, less than one mutation on average (Table 3). The estimated values for U_I (per haploid) closely reflect the changes of mean and variances, with, for example, early productivity and relative fitness showing significantly higher rates for detectable mutations than the other life-history traits. The average effects for these detectable mutations are relatively large, in the range of 15–24%.

Under the ML analysis with the assumption of equal mutation effects, estimates of U_I (s) for total productivity and longevity are somewhat higher (lower) than under BM, but are lower (higher) for early productivity. Under ML, higher estimates for numbers of mutations with correspondingly lower estimates for average effects tend to occur if there is variability among mutation effects (Keightley 1998). To further investigate properties of the distribution of mutational effects, univariate ML analyses were carried out under the assumption that mutation effects are gamma distributed (Table 4). Profile likelihoods were computed as functions of the distribution parameters (β and $\bar{s}$) and U_I. For all traits, the best-fitting gamma distribution is the limiting case of equal effects ($\beta \to \infty$), and strongly leptokurtic distributions ($\beta \to 0$) are excluded for total and early productivity and relative fitness. Any gamma distribution is plausible for longevity, since the trait is highly environmentally

TABLE 1

ANOVA for control and EMS lines

Trait	Treatment	Source of Variation	d.f.	MS	*F*-ratio
Productivity	CON	Assay	2	61537	25.37***
		Measurer	2	359	0.15
		Assay–measurer	4	7393	3.05*
		Line	39	2044	0.84
		Residual	309	2426	
	EMS	Assay	2	25096	7.29***
		Measurer	2	972	0.28
		Assay–measurer	4	7956	2.31
		Line	55	47713	13.87***
		Residual	431	3440	
Early productivity	CON	Assay	2	124193	63.37***
		Measurer	2	4202	2.14
		Assay–measurer	4	10042	5.12***
		Line	39	1593	0.81
		Residual	309	1960	
	EMS	Assay	2	35963	34.37***
		Measurer	2	1947	1.86
		Assay–measurer	4	6110	5.84**
		Assay–line	110	2342	2.24*
		Measurer–line	110	2352	2.25*
		Assay–measurer–line	193	1689	1.61
		Line	55	25692	24.55***
		Residual	18	1046	
Late productivity	CON	Assay	2	11133	10.95***
		Measurer	2	4224	4.16*
		Assay–measurer	4	7484	7.36***
		Line	39	807	0.79
		Residual	309	1017	
	EMS	Assay	2	1513	1.08
		Measurer	2	1898	1.36
		Assay–measurer	4	2121	1.52
		Line	55	10792	7.73***
		Residual	431	1397	
Longevity	CON	Assay	2	147.4	18.93***
		Measurer	2	56.17	7.21***
		Assay–measurer	4	21.33	2.74*
		Line	39	9.01	1.16
		Residual	309	7.79	
	EMS	Assay	2	102.5	7.50***
		Measurer	2	9.45	0.69
		Assay–measurer	4	38.78	2.84*
		Line	55	42.01	3.07***
		Residual	422	13.67	
Relative fitness	CON	Assay	2	5.693	63.29***
		Measurer	2	0.641	7.13***
		Assay–measurer	4	0.698	7.75***
		Line	39	0.0745	0.83
		Residual	309	0.0900	
	EMS	Assay	2	1.363	52.60***
		Measurer	2	0.172	6.65**
		Assay–measurer	4	0.322	12.43***
		Assay–line	110	0.0825	3.18**
		Measurer–line	110	0.0747	2.88**
		Assay–measurer–line	193	0.0608	2.34*
		Line	55	0.733	28.27***
		Residual	18	0.0259	

MS, mean square. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

TABLE 2

Means and variances for the life-history traits in control and EMS lines and scaled effects of the EMS treatment, along with their standard errors

Trait	M_{CON}	M_{EMS}	$\Delta M/M$	h^2_M	CV_M
Total productivity (worms)	248 ± 2.5	163 ± 9.9	0.34 ± 0.039	1.04 ± 0.22	0.20 ± 0.018
Early productivity (worms)	192 ± 2.2	97.5 ± 7.2	0.49 ± 0.042	0.72 ± 0.13	0.20 ± 0.016
Late productivity (worms)	56.6 ± 1.5	65.7 ± 4.7	−0.16 ± 0.089	0.53 ± 0.13	0.41 ± 0.052
Longevity (days)	13.4 ± 0.16	12.2 ± 0.29	0.090 ± 0.023	0.20 ± 0.10	0.093 ± 0.023
Relative fitness	1.00	0.46 ± 0.039	0.54 ± 0.039	0.45 ± 0.092	0.20 ± 0.018

sensitive, and there is consequently little information on shape. In the cases of productivity and longevity, the combination of higher U_I estimates under ML than BM and a better fit for the equal-effects model than the gamma distribution suggests that there may be discontinuities in the distribution of mutational effects that are not well captured by assuming a gamma distribution of mutation effects in the analysis. However, it is difficult to extract a great deal of information on shape, even in experiments more highly replicated than the present one, since there is a strong tendency toward high sampling covariances between the parameters (Keightley 1998).

Genetic correlations among line means: Estimates of genetic correlation coefficients along with bootstrap standard errors are shown in Table 5. The estimated genetic correlation between early productivity and relative fitness is close to 1. Mutational correlations are strong and positive; the weakest is between relative fitness and late productivity. There is no appreciable difference between the early productivity:longevity genetic correlation and the late-productivity:longevity correlation, so at this coarse level there is no evidence for a trade-off. Bivariate plots of line means for longevity with early and late productivity reveal interesting patterns (Figure 3). There is no evidence for lines that have

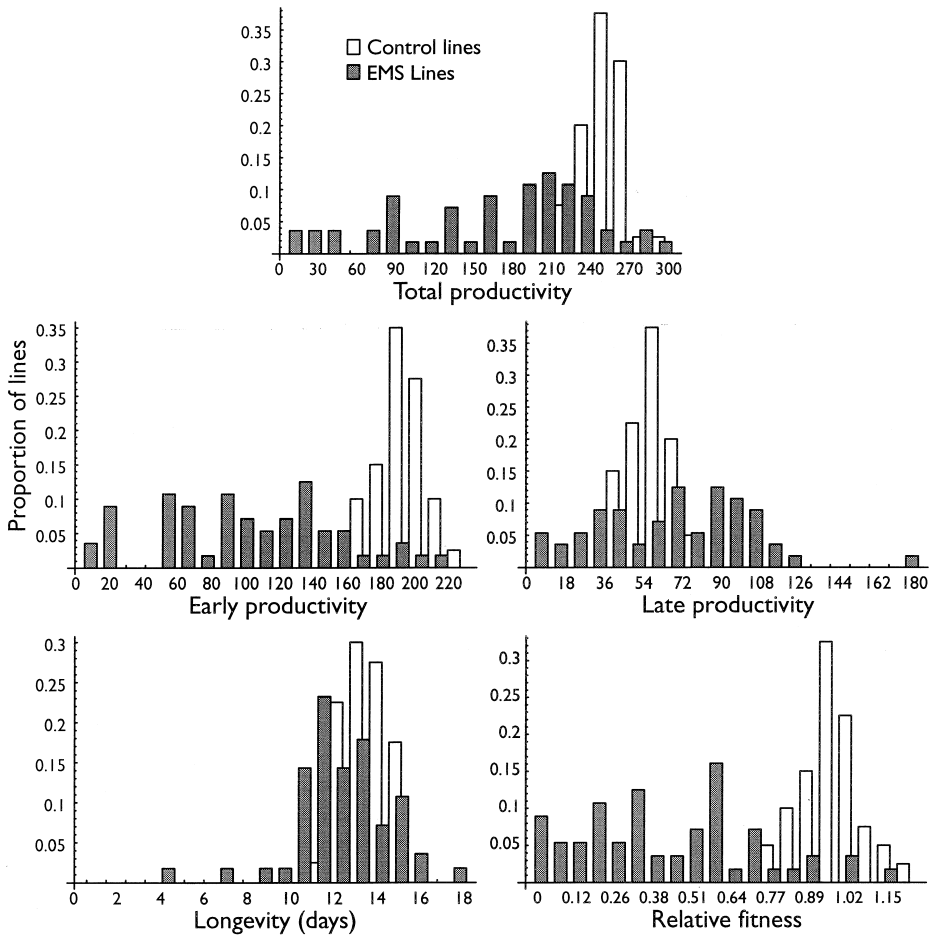

Figure 1.—Distributions of control and EMS line means for the life-history traits.

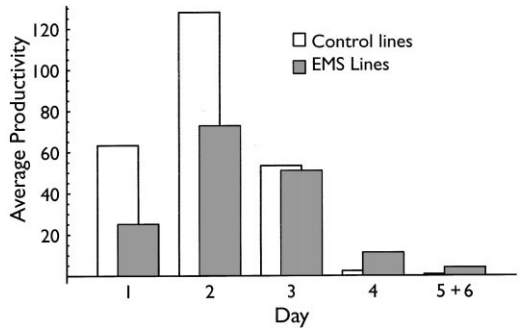

FIGURE 2.—Daily mean reproductive output for control and EMS lines during the reproductive period.

decreased longevity and increased early productivity, as might be expected under the pleiotropic theory for the evolution of aging (Figure 3A; in fact, there are no lines with significantly increased early productivity). Other lines show evidence of trade-offs: there are many lines with reduced longevity and increased late productivity (Figure 3B). There is one line with significantly increased longevity and increased late productivity (see below). This line also has significantly reduced early productivity.

Bivariate analysis—simulation results: To verify the bivariate ML computer program, simulations were carried out to estimate parameter values for cases in which the simulated values were known. To simplify the interpretation of the results and to reduce the dimensionality of the parameter space that needed to be searched, the shape parameter of the bivariate distribution that was assumed in the analysis was the same as that simulated. Thee parameters to be estimated were U, ρ, the mean mutational effects for the traits, and the residual environmental variances and covariance. Means and standard deviations of estimates of U and ρ from a limited number of these computer-intensive simulations are shown in Table 6. The mean estimates do not differ significantly from simulated values, implying that the estimation procedure is behaving reasonably well. However, although the "correct" bivariate distribution is assumed, it is notable that sampling variances of ρ are relatively high.

Bivariate analysis—*C. elegans* life-history traits: We carried out the bivariate ML analysis to infer properties of the bivariate distribution of mutation effects for two pairs of traits: total productivity and longevity, and total productivity and relative fitness (Table 7). Likelihood was evaluated for a series of models with different gamma distribution shape parameters (β). The main parameter of interest was ρ, the mutational correlation. The best-fitting bivariate gamma distributions have $\beta \simeq 1.5$ in the case of productivity:longevity and ~ 8 in the case of productivity:relative fitness, but likelihood surfaces as a function of β are very flat. Likelihood drops sufficiently to reject the equal-effects model ($\beta \rightarrow \infty$) in both cases, although this is the best-fitting univariate distribution (Table 4). The best estimates for ρ are ~ 0.1 (productivity:longevity) and 0.2 (productivity:relative fitness), but confidence limits on ρ within β models are extremely wide. Interestingly, mutational distributions with zero correlation fit the data nearly as well as the best-fitting distribution; the reasons for this are explained in the next section. ML estimates for U_I with the bivariate model are $\hat{U}_I = 2.6$ (productivity:relative fitness) and $\hat{U}_I = 2.2$ (productivity:longevity); compare Table 3. The bivariate estimates probably underestimate the rate for mutations that are deleterious in natural conditions by at least 20-fold (DAVIES *et al.* 1999).

Relationship between genetic correlation and correlation of mutational effects: The bivariate ML analysis of the *C. elegans* life-history traits gave estimates for ρ that are lower than the genetic correlation parameter, r_G. The traits are strongly and significantly genetically correlated (Table 5), but profile likelihoods also imply that a zero value for ρ can plausibly explain the data (Table 7). Paradoxically, it seems that the correlation of the "underlying" mutational distribution can be very different from the genetic correlation of line means: a high genetic correlation does not necessarily imply a high underlying mutational distribution correlation. The explanation seems to be that genetic correlation is generated because different lines carry different numbers of mutations; lines that carry the highest numbers of mutations tend to be extreme for both traits, even if the mutational effects are uncorrelated. This is analogous to the "apparent" (*i.e.*, correlated) stabilizing selection that can be generated with a pleiotropic model of mutation effects on a quantitative trait and fitness (BARTON 1990; KEIGHTLEY and HILL 1990; see also ROBERTSON 1967). This is illustrated graphically in Figure 4.

As long as all mutations reduce each trait, the actual relationship between r_G and ρ turns out to be a simple function of the gamma distribution shape parameter and ρ. As long as $\rho > 0$, r_G will always be $> \rho$. The genetic variance of trait X as a function of U, α_X, and β_X is

$$\mathrm{var}(X) = \frac{U\beta_X(\beta_X + 1)}{\alpha_X^2}, \tag{4}$$

the mean is

$$E(X) = \frac{U\beta_X}{\alpha_X}, \tag{5}$$

and the expected cross product is

$$E(XY) = \frac{U\beta_X^{1/2}\beta_Y^{1/2}\rho + \beta_X\beta_Y(U + U^2)}{\alpha_X\alpha_Y}. \tag{6}$$

The genetic correlation is

$$r_G = \frac{\rho + (\beta_X\beta_Y)^{1/2}}{[(\beta_X + 1)(\beta_Y + 1)]^{1/2}}, \tag{7}$$

so the relationship between r_G and ρ depends on the

TABLE 3

BM and ML estimates of U_I per haploid and s from univariate analysis, under a model of equal mutation effects

	BM		ML	
Trait	$\hat{U}_I$	$\hat{s}$	$\hat{U}_I$	$\hat{s}$
Total productivity	1.40 ± 0.35	0.24 ± 0.037	1.61 ± 0.30	0.22 ± 0.028
Early productivity	3.11 ± 0.88	0.16 ± 0.027	2.06 ± 1.06	0.23 ± 0.058
Longevity	0.50 ± 0.62	0.19 ± 0.10	0.80 ± 0.46	0.13 ± 0.045
Relative fitness	3.55 ± 1.07	0.15 ± 0.031	3.60 ± 1.31	0.15 ± 0.053

The trait late productivity was not analyzed, since there is evidence that mutations increase and decrease the trait with nearly equal probability (Figure 1).

relative magnitude of the β's. However, r_G will always be $>\rho$.

Under the assumption that the β's are the same for each trait (as assumed in the bivariate analysis), the genetic correlation is

$$r_G = \frac{\rho + \beta}{\beta + 1}. \quad (8)$$

This shows that if $\beta \gg 1$, the genetic correlation tends toward 1 for any value of ρ, because the correlation is wholly induced by different individuals having different numbers of mutations. If the distribution is leptokurtic ($\beta \to 0$), ρ and r_G become the same, since the genetic correlation is generated by a few individuals carrying mutations of very large effect. If the mutational distribution has moderate kurtosis (for example, the bivariate exponential distribution, $\beta = 1$), the genetic correlation is 0.5 even if there is zero correlation between mutation effects. Note that these results depend on the assumption of unidirectional mutational effects: if the distributions are symmetrical about zero, the genetic correlation would be zero irrespective of the values of ρ and β.

DISCUSSION

Effects of EMS on life-history traits: The effects of mutagenesis on the different life-history traits were compared in a number of different ways. In terms of scaled changes of mean phenotype, early productivity and relative fitness are substantially larger mutational targets than total or late productivity. Our results strongly suggest that longevity is much less affected by mutation accumulation than the productivity traits or relative fitness. Most other published data also suggest that longevity is a small mutational target in relation to other life-history traits, since directional effects on mean longevity in mutation-accumulation experiments have been difficult to detect. In a spontaneous mutation-accumulation experiment in *C. elegans* (Vassilieva and Lynch 1999), there is little indication of a mutational decay for longevity after ≃200 generations (M. Lynch and L. Vassilieva, personal communication). In addition, a mutation-accumulation experiment over 60 generations with the same *C. elegans* strain did not reveal a significant directional change (Keightley and Caballero 1997). In *Drosophila melanogaster*, there is also information on the effects of spontaneous mutation accumulation on longevity (Pletcher *et al.* 1999). After 47 generations of mutation accumulation, there was significant mutational variation for longevity in both sexes, but little sign of directional mutational bias. An EMS mutagenesis experiment in *D. melanogaster* in which life-history traits were assayed has also been reported (Keightley and Ohnishi 1998). For the composite trait, fertility ×

TABLE 4

ML estimates and support limits for U_I, $\bar{s}$, and β obtained by univariate analysis under a model of gamma-distributed mutation effects

	U_I			$\bar{s}$			β		
Trait	MLE	Lower	Upper	MLE	Lower	Upper	MLE	Lower	Upper
Total productivity	1.6	1.3	3.3	0.22	0.10	0.23	→∞	0.7	→∞
Early productivity	2.1	1.7	4.3	0.23	0.11	0.24	→∞	3.3	→∞
Longevity	0.80	0.34	→∞	0.13	→0	0.22	→∞	→0	→∞
Relative fitness	3.6	2.3	7.4	0.15	0.075	0.23	→∞	1.2	→∞

Support limits are parameter values that give a drop in natural log-likelihood of 2 from the ML, while likelihood with respect to all the other parameters in the model is maximized. MLE, ML estimate.

TABLE 5

ASREML estimates of genetic and environmental correlation coefficients

	Total productivity	Early productivity	Late productivity	Longevity	Relative fitness
Total productivity		0.90 (0.030)	0.74 (0.072)	0.58 (0.16)	0.87 (0.033)
Early productivity	0.77 (0.035)		0.38 (0.16)	0.49 (0.13)	0.99 (0.006)
Late productivity	0.47 (0.045)	−0.00 (0.099)		0.47 (0.21)	0.32 (0.14)
Longevity	0.15 (0.073)	0.07 (0.076)	0.14 (0.060)		0.52 (0.14)
Relative fitness	0.59 (0.074)	0.88 (0.016)	−0.32 (0.048)	0.047 (0.072)	

Genetic (environmental) correlations are above (below) the diagonal. Standard errors (in parentheses) were obtained by bootstrapping the data by line, 100 times.

hatchability, which is closest to total productivity, the scaled change in mean between controls and treated lines was ~25%, compared to a 17% change for longevity. The effect of EMS on mean longevity in the flies was therefore larger, in comparison to other life-history traits, than we have observed in *C. elegans*.

Alternative measures of mutational target size are the estimated numbers of mutations affecting the traits, obtained by BM or ML methods. BM estimates are effective numbers of major effect mutations and are biased downward if there is variability among mutational effects. Maximum likelihood can partly overcome this bias by assuming that mutations follow some distribution whose shape can be estimated, but estimates of numbers of mutations are often unbounded (Keightley 1998). For *C. elegans*, there are independent estimates of the number of mutations induced by EMS mutagenesis (Anderson 1995). A 50 mM treatment generates ~200 point mutations per genome, of which ~50 change an amino acid in a protein-coding gene. Protein-coding genes are evolutionarily highly constrained in *C. elegans* (Stenico *et al.* 1994; Shabalina and Kondrashov 1999); the majority of amino acid changes are therefore deleterious in natural conditions. Furthermore, there may be as many selectively constrained sites outside of genes as there are within coding sequences (Shabalina and Kondrashov 1999). Therefore, the estimates for numbers of mutations we obtained from the analyses of the phenotypic distributions may be >10 times too low (Davies *et al.* 1999). It is perhaps most logical to regard all traits as having been affected by the same number of mutations, as the bivariate analysis does, but there were different marginal distributions of mutational effects. Comparing mutational target sizes of life-history traits solely on the basis of estimated numbers of mutations from phenotypic assays is therefore problematical.

The analyses to infer mutation rates and effects assumes Poisson-distributed mutation numbers among lines. If dosage variation leads to clustering of mutations, then this will lead to underestimation of U and overestimation of $\bar{s}$ (Keightley and Ohnishi 1998). However, this is probably unimportant in the present case for the following reasons: first, mutagenized worms were from a synchronous culture and were at the same stage of development. Second, there was one mutagenesis treatment, and all progenitor worms were exposed in the same vial for identical periods of time. And third, the estimates of average mutation effects and distribu-

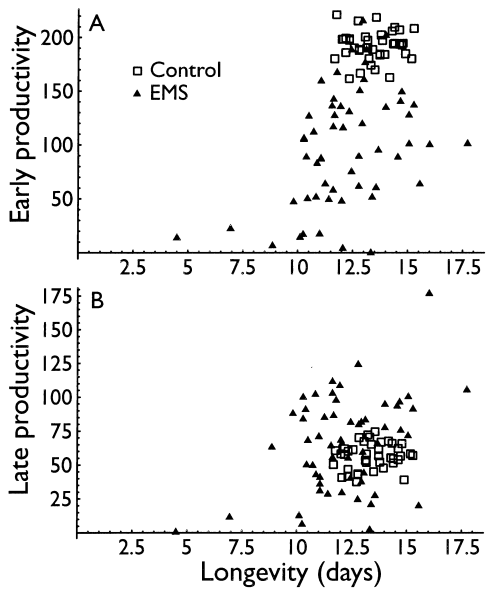

Figure 3.—Line means for longevity plotted against line means for early (A) and late (B) productivity.

TABLE 6

Simulation results from bivariate ML analysis

Simulated values		Estimates			
U	ρ	$\hat{U}$	(SD)	$\hat{\rho}$	(SD)
0.5	0.5	0.50	(0.12)	0.48	(0.20)
1.0	0.3	1.03	(0.21)	0.34	(0.17)
1.0	0.7	1.03	(0.20)	0.71	(0.10)

The simulations involved 100 MA and 100 control lines, with the ratio $V_G/V_E = 5$ or 10, and zero environmental covariance. There were 40 replicates per parameter set combination.

TABLE 7

Estimates of the mutational correlation parameter ρ from bivariate ML analysis of *C. elegans* EMS lines data, assuming a range of shape parameters, β

		2 Log *L* support limits for ρ		
β	$\hat{\rho}$	Lower	Upper	Log *L* difference
Total productivity: longevity				
0.25	0.51	0.07	0.84	−1.0
0.5	0.44	0	0.81	−0.5
1	0.27	0	0.75	−0.1
1.5	0.11	0	0.70	0
2	0	0	0.64	−0.0
3	0	0	0.61	−0.5
4	0	0	0.61	−1.0
8	0	0	0.86	−2.5
→∞	Undefined	—	—	−3.8
Total productivity: relative fitness				
0.25	0.71	0.51	0.85	−2.8
0.5	0.68	0.46	0.84	−1.6
1	0.61	0.32	0.81	−0.6
1.5	0.55	0.18	0.78	−0.2
2	0.47	0.025	0.74	−0.0
4	0.32	0	0.68	−0.0
8	0.17	0	0.60	0
12	0	0	0.39	−1.9
→∞	Undefined	—	—	−7.5

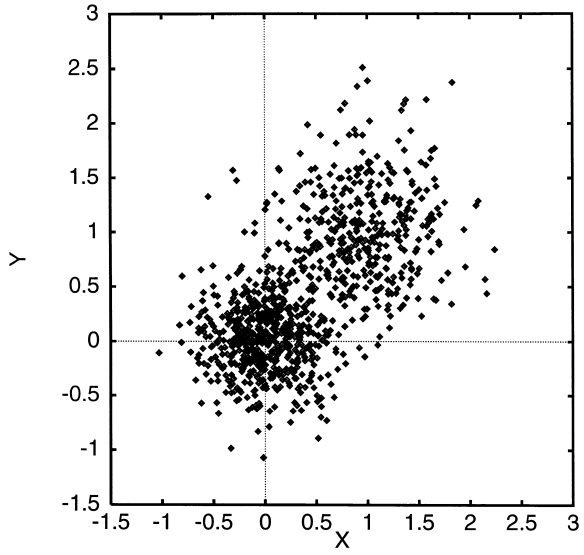

FIGURE 4.—Extreme case of strong apparent genetic correlation generated in the absence of correlation between mutational effects. There are 1000 individuals having 0.5 mutations, on average, drawn from a bivariate gamma distribution with shape parameter 8 and mean 1 for each trait. The genetic correlation is 0.89.

tion parameters are similar to those estimated in a spontaneous mutation accumulation (MA) carried out under similar conditions (KEIGHTLEY and BATAILLON 2000); spontaneous mutations are usually assumed to occur as a Poisson process.

Correlations between traits: The genetic correlation estimates between life-history traits are strong and estimated relatively precisely (Table 5), but what does this tell us about the underlying genetics of the traits? Genetic correlations can be induced either by linkage disequilibrium or pleiotropy (FALCONER and MACKAY 1996). In mutation-accumulation lines, a linkage effect also occurs because different lines carry different sets of mutations whose effects may differ. This can generate genetic correlations between traits in the absence of any correlation due to pleiotropy. We show that under an additive model with unidirectional mutational effects, the genetic correlation is a function of the parameters of the joint distribution of effects of mutations on the two traits, but is independent of the mean number of mutations per line (Equation 8). Genetic correlations are therefore not expected to change in a mutation-accumulation experiment as additional mutations accumulate over time if mutational effects are additive. If mutational effects follow a bivariate distribution in which the coefficients of kurtosis for the marginal distributions are small (*e.g.*, a bivariate gamma distribution with large β), the genetic correlation is induced solely by lines carrying different numbers of mutations. Since the univariate estimates of β do not rule out the possibility that the detectable mutations for life-history traits in *C. elegans* have a platykurtic distribution (Table 4), it is therefore possible that the mutational correlations are small, in spite of the strong genetic correlations. A more direct approach to infer the underlying mutational correlation is to explicitly estimate a mutational correlation parameter (ρ) by a bivariate analysis. We have developed a procedure to carry out such an analysis. The analysis is difficult to carry out because a large number of parameters need to be estimated simultaneously, and likelihood maximization can present problems. However, the results of ML analyses of simulated data suggest that the procedure functions correctly. The results from bivariate analysis of the *C. elegans* EMS data are disappointing in that the plausible range for ρ is very large. For example, in the case of longevity:productivity, the best estimate for ρ is ∼0.1 (*i.e.*, smaller than the genetic correlation, as expected), but the upper limit is >0.8 (Table 7). Thus, the empirical analyses suggest that the mutational correlation parameter is extremely difficult to estimate with any precision from a mutation-accumulation experiment even if the genetic correlation is precisely estimated. Estimates of the correlation parameter are correlated with β (see Equation 8), which itself is strongly correlated with the estimated number of mutations and their mean effect (KEIGHTLEY 1998). The confounding effect may be partly overcome if the number of mutation events each line carries is known or can be directly estimated, as can be the case with transposable element insertional mutagenesis (HILL 1992; MACKAY *et al.* 1992).

Mutational effects on longevity: It is surprising that

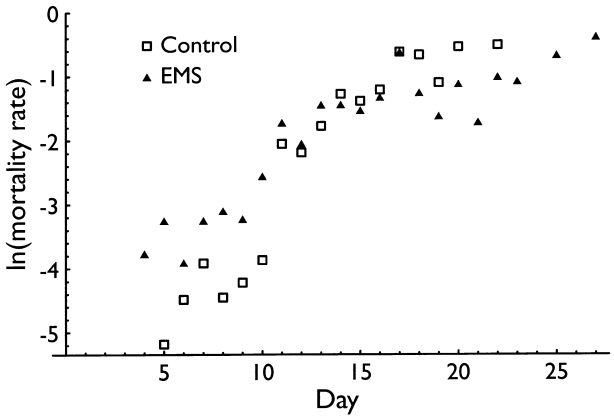

FIGURE 5.—Age-specific pattern of mortality calculated separately for EMS and control lines (plotted on a natural log scale).

after a large dose of new mutations (equivalent to 500–1000 generations of spontaneous mutation accumulation; DAVIES *et al.* 1999), the change in mean longevity between the EMS and control lines was <10%. Of the 56 lines, there were 5 with significantly reduced and 1 with significantly increased longevity. There is therefore some evidence for bidirectional effects of mutations on longevity. There is no evidence for trade-offs between early productivity and longevity. Furthermore, the estimates of genetic correlations between longevity and early and late productivity are essentially the same. However, the possibility that a small number of antagonistically pleiotropic mutations are present (or that such mutations have already become fixed by selection) cannot be ruled out.

In both EMS and control populations, there appears to be a flattening of mortality curves (Figure 5), a phenomenon that has been repeatedly documented, but that has two potential explanations (CHARLESWORTH and PARTRIDGE 1997). It could be a real effect brought about by a general reduction in mortality rate with age, or it could be induced by heterogeneity in survivorship curves (VAUPEL *et al.* 1979). Surprisingly, the EMS lines seem to show a stronger flattening of the mortality curve than the controls, and have lower average mortality late in life. This effect could also have been brought about by a negative relationship between fertility and longevity (CHARLESWORTH and PARTRIDGE 1997), since the EMS lines have much lower total productivity than controls. Distinguishing between these various causes for the shape of the mortality curve will require much more highly replicated experiments in which mortality curves for individual lines can be estimated and perhaps experiments involving males for which access to hermaphrodites can be eliminated.

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