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THE FITNESS EFFECTS OF SPONTANEOUS MUTATIONS IN *CAENORHABDITIS ELEGANS*

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Abstract.—Spontaneous mutation to mildly deleterious alleles has emerged as a potentially unifying component of a variety of observations in evolutionary genetics and molecular evolution. However, the biological significance of hypotheses based on mildly deleterious mutation depends critically on the rate at which new mutations arise and on their average effects. A long-term mutation-accumulation experiment with replicate lines of the nematode *Caenorhabditis elegans* maintained by single-progeny descent indicates that recurrent spontaneous mutation causes approximately 0.1% decline in fitness per generation, which is about an order of magnitude less than that suggested by previous studies with *Drosophila*. Two rather different approaches, Bateman-Mukai and maximum likelihood, suggest that this observation, along with the observed rate of increase in the variance of fitness among lines, is consistent with a genomic deleterious mutation rate for fitness of approximately 0.03 per generation and with an average homozygous effect of approximately 12%. The distribution of mutational effects for fitness appears to have a relatively low coefficient of variation, being no more extreme than expected for a negative exponential, and for one composite fitness measure (total progeny production) approaches constancy of effects. These results are derived from assays in a benign environment. At stressful temperatures, estimates of the genomic deleterious mutation rate (for genes expressed at such temperatures) is sixfold lower, whereas those for the average homozygous effect is approximately eightfold higher. Our results are reasonably compatible with existing estimates for flies, when one considers the differences between these species in the number of germ-line cell divisions per generation and the magnitude of transposable element activity.

Key words.—*Caenorhabditis elegans*, deleterious mutation, dominance, fitness, life-history characters, mutation load, mutation rate, quantitative traits.

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Spontaneous mutation to mildly deleterious alleles has recently emerged as a leading and potentially unifying component of a variety of observations in evolutionary genetics and molecular evolution. Population-level phenomena thought to be explained include the magnitude of inbreeding depression and standing levels of genetic variance for fitness-related characters, the evolution of mating systems, and the extinction of small populations (Lande and Schemske 1985; Charlesworth and Charlesworth 1987; Kondrashov 1988; B. Charlesworth 1990; Lynch et al. 1995). Numerous features of genome evolution are also thought to be consequences of the recurrent input of very slightly deleterious mutations (Bulmer 1991; Ohta 1992; Rice 1994; Lynch and Blanchard 1998; Force et al. 1999). In addition, the case has been made that the fate of our own species may be influenced by the inevitable accumulation of mutational load resulting from relaxed selection in a cultural setting with advanced medical procedures (Muller 1950; Kondrashov 1995; Crow 1997; Lynch et al. 1999). The extent to which mutation actually plays a role in any of these phenomena depends critically on the rate at which deleterious mutations arise, the distribution of their fitness effects, and their degree of dominance. For example, deleterious mutation is unlikely to be a significant risk of extinction of many natural populations unless the number of mutations arising per genome is relatively high (greater than about 0.1 per generation) and a substantial fraction of them has relatively small effects (on the order of a few percent reduction in fitness per mutation or smaller; Lynch et al. 1995; Schultz and Lynch 1997).

Although a recurrent rate of genomic fitness loss via spontaneous mutation on the order of 0.5% to 1.0% per generation seems to have fairly broad empirical support in the fly *Drosophila melanogaster* (see review in Lynch et al. 1999), there is still uncertainty about the magnitude of the components leading to this decline. When analyzed with a statistical method developed by Bateman (1959), early results from mutation-accumulation experiments with flies (e.g., Mukai et al. 1972; Ohnishi 1977) led to the suggestion that the minimum genomic deleterious mutation rate in this species is approximately 0.6 per generation and that the average selection coefficient is less than 3% in the heterozygous state. However, these conclusions have recently been challenged on both empirical and statistical grounds, with speculation also arising that the rapid decline in fitness in the Mukai/Ohnishi lines may have been an illusion resulting from the adaptive evolution of control chromosomes (Keightley 1996; García-Dorado 1997; Caballero and Keightley 1998; García-Dorado et al. 1998). Fry et al. (1999) performed an experiment similar in design to those of Mukai and Ohnishi, but concluded that the rapid rate of buildup of mutational load is a consequence of a lower genomic rate of mutation (perhaps as low as 0.1 per individual) and higher average mutational effects, and minimum-distance analyses applied to the earlier data have led to estimates of the genomic deleterious mutation rate as low as 0.01 (García-Dorado 1997; García-Dorado et al. 1998).

The idea that the genomic deleterious mutation rate in flies is as low as 0.1 or even 0.01 is far from resolved. First, observed levels of inbreeding depression and genetic variation for fitness-related traits as well as known genomic rates of transposition and nucleotide substitution appear to be consistent with the classical interpretation of the Mukai and Ohn-

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ishi data (Lynch et al. 1999). Second, the genomic mutation rate to lethals is known to be on the order of 0.01 to 0.05 (Crow and Simmons 1983; Fry et al. 1999), and it seems unlikely that this would exceed the rate of origin of detectable mutations with milder effects. Third, the study by Fry et al. (1999) seems to have ruled out the possibility of control chromosome evolution, thereby weakening (although not eliminating) a common, but unsubstantiated, criticism of the earlier fly studies. Fourth, maximum-likelihood analyses of the data from *Drosophila* mutation-accumulation experiments are generally unable to reject a model with an extremely high rate of origin of mutations with extremely small effects (Keightley 1996, 1998; Fry et al. 1999).

Some of the differences among results on *Drosophila* may be a consequence of real variation among strains in the mutation rate and/or distribution of mutational effects. There can, for example, be large differences among individuals in the number of transposable elements, even within populations (Charlesworth and Langley 1989). Given that the average number of transposition events is approximately 0.8 per generation in *Drosophila* (Nuzhdin and Mackay 1995), and given that this rate accelerates with the number of mobile elements per genome (Nuzhdin et al. 1996), such variation is likely to translate into substantial among-strain differences in deleterious mutational properties. It is also possible that the variation observed among different fly studies is a simple consequence of undefined differences in experimental conditions.

These problems aside, a more general question remaining is the extent to which the mutational properties observed in *Drosophila* are representative of those in other species. The only other metazoans for which estimates of the properties of deleterious mutation have been obtained are the microcrustacean *Daphnia pulex* (Deng and Lynch 1997; Lynch et al. 1998) and the nematode *Caenorhabditis elegans* (Keightley and Caballero 1997; Vassilieva and Lynch 1999). For *Daphnia* the results do not differ greatly from the classical Mukai/Ohnishi interpretation for flies. However, the results from *C. elegans* suggest a rather different situation—the decline in the mean of fitness-related characters is only about 0.03% to 0.3% per generation, estimates of the genomic deleterious mutation rate are on the order of 0.005 to 0.05 per individual, and estimates of the average deleterious homozygous effects of mutations are in the range of 5% to 25%.

The previous studies with *C. elegans* involved only 50 to 60 generations of mutation accumulation, and the statistical power was limited (Keightley and Caballero 1997; Vassilieva and Lynch 1999). If the true genomic mutation rate per fitness character is near the upper end of the estimated range, 0.05 per generation, then at the time of the final assays, the average experimental line in these studies would be expected to contain only about one new mutation, and about a quarter of the lines would be expected to be mutation free. If the rate is as low as 0.005, then on the order of 90% of the lines would have been mutation free at the time of the final assay. Thus, the fact that neither study was able to detect a significant rate of change in total progeny production may have been a simple consequence of insufficient time for the detection of mutations with small effects. For that reason, we have extended our previous study to 214 generations of mutation accumu-

lation. Here, we demonstrate a significant rate of deterioration of all fitness-related traits, while also corroborating the previous suggestion that the genomic mutation rate in nematodes is lower than earlier estimates in flies. In addition, we evaluate the extent to which this interpretation depends on the conditions under which individuals are assayed. Finally, we present results that suggest that spontaneous mutations in nematodes have average effects that are roughly additive to partially recessive in the heterozygous state.

MATERIALS AND METHODS

Base Strain, Line Maintenance, and Assay Conditions

The basic procedures underlying this study have been outlined in detail in Vassilieva and Lynch (1999), so we only provide a cursory overview of the approaches. The mutation-accumulation experiment was initiated with a single individual derived from the Bristol-N2 strain of *C. elegans* (obtained directly from the *Caenorhabditis* Genetic Center, St. Paul, MN) taken to a very high degree of homozygosity by repeated rounds of self-fertilization and single-progeny descent. From the F₃ descendants of a single individual, 100 mutation-accumulation lines were initiated, while many thousands of remaining progeny were kept frozen as controls, stored at -80°C, as described in Lewis and Fleming (1995).

Individual nematodes were cultured at 20°C and maintained on medium (60 × 15 mm) petri dishes containing NGM agar seeded with a standard suspension of *Escherichia coli* strain OP50 as a food source. To minimize the efficiency of natural selection against new mutations, each of the lines was propagated across generations as a single random worm. As a rule, to avoid selection for early or late reproduction, offspring produced in the middle of the parental reproductive period were used in the transfers. To prevent accidental losses of the experimental lines, individuals from two previous generations were maintained at 15°C as potential backups.

The experimental lines were assayed with parallel controls on eight occasions, at which times line divergence had proceeded for an average of 0, 7, 20, 30, 49, 89, 119, 163, and 214 generations. To ensure that maternal and grandmaternal environmental effects did not contribute to the among-line component of variance, prior to each assay each line was divided into five replicates (four in the third assay), all of which were then transferred as single individuals for two generations, with single third-generation descendants from each replicate being employed in the actual assay (Lynch 1985). At each assay, the same procedure of replication followed by two generations of line transfers was applied to 20 animals taken from the frozen control stock, resulting in 100 control individuals (five descendants from each of 20 thawed animals). The complete series of assays from generations 0 to 214 was performed at 20°C, a near optimal temperature for the growth of *C. elegans*. In addition, at generations 163 and 214, we performed a parallel assay at 12°C along with the controls. Except for the temperature change in the assay generation, these assays were identical in all respects to those run at 20°C.

Characters Assayed

The life-history characters assayed in this study are identical to those reported on previously (Vassilieva and Lynch 1999): (1) productivity, the total number of viable progeny produced over the first four days of reproduction (first 12 days for the 12°C assays, because the generation time is extended at this temperature); (2) survival to maturity, a 0/1 variable denoting whether the individual produced any viable progeny; (3) longevity, number of days until death; (4) intrinsic rate of increase, r , the rate of exponential growth (days^{-1}) expected for a line once the stable age distribution has been attained; (5) a measure of the rate of convergence to the stable age distribution (ϕ , also in units of days^{-1}); and (6) the generation rate, the reciprocal of the mean age of reproduction for the members of a line (days). Technical details regarding the computation of these measures can be found in Vassilieva and Lynch (1999).

Rates of Change of Means and Variances of Phenotypes

The assays reported on here were performed over a period of approximately three years. During that time, a systematic change in laboratory conditions could have resulted in a temporal trend in the phenotypic distributions of the mutation-accumulation lines unassociated with genetic changes. In addition, 27 of the original 100 lines went extinct over the course of the experiment, and it is possible that phenotypic changes associated with mutations in such lines were related to the extinction process. To minimize the bias in our analyses that might result from such effects, the mean phenotypes of the mutation-accumulation lines were corrected in two ways prior to the analysis of the data.

First, to factor out any temporal trend due to environmental change, the grand means of all of the mutation-accumulation lines were jointly regressed on the parallel control means and on generation number (Muir 1986; Lynch 1988). The partial regression coefficient involving the control means was significant only in the case of longevity and generation rate. For these two traits, the corrected means (\bar{z}') were obtained as

$$\bar{z}'(t) = \bar{z}(t) - b_c[\bar{z}_c(t) - \bar{z}_c], \quad (1)$$

where $\bar{z}(t)$ and $\bar{z}_c(t)$ denote the observed means for the mutation-accumulation lines and the controls in generation t , \bar{z}_c is the average control mean over all assays, and b_c is the partial regression coefficient involving the control mean. For the remaining characters, the temporal changes in the control means were very slight, nonsignificant, and uncorrelated with the means of the experimental lines, as will be shown below.

Second, to account for bias resulting from selective line extinctions, each assay was reanalyzed using only the lines that survived to be included in the next assay. An estimate of the change in the mean phenotype between assays i and $i + 1$ caused by differential survival of lines was obtained as

$$\Delta\bar{z}(i) = \bar{z}_s(i) - \bar{z}(i), \quad (2)$$

where $\bar{z}_s(i)$ is the mean of the lines present at assay i that also survived to the following assay. The cumulative amount of change in the mean caused by all selective mortality up to assay j was then estimated by

$$\Delta_{j-1} = \sum_{i=1}^{j-1} \Delta\bar{z}(i), \quad (3)$$

and the mean phenotype at assay j corrected for all previous selective change was defined as

$$\bar{z}'_s(j) = \bar{z}(j) - \Delta_{j-1}, \quad (4)$$

where the observed mean in assay j , $\bar{z}(j)$, was corrected by use of equation (1) in the case of longevity and generation rate.

Identical procedures to those noted above were used to correct the observed among-line variance estimates for the mutation-accumulation lines. In this case, productivity and longevity needed to be corrected for a correlated trend in the control variance.

Estimates of the Mutation Rate and Average Homozygous Effect

Standard least-squares regression procedures were used to estimate the rate of change in the mean phenotypes across lines (R_m) and the rate of increase in the among-line variance (V_b). Equating half the rate of increase in the among-line variance to the mutational variance (V_m) of a trait and the average estimate of the within-line variance to the environmental variance (V_e), the mutational heritability (h_m^2) was estimated as the ratio of the two, after correcting for the bias due to sampling error (Vassilieva and Lynch 1999). An expression for the standard error of h_m^2 is given in our previous paper.

For each character, we derived downwardly biased estimates of the diploid genomic deleterious mutation rate and upwardly biased estimates of the average homozygous effects of mutations, using the formulae presented by Bateman (1959) and Mukai (1964),

$$U_{min} = \frac{2(R_m)^2}{V_b} \quad \text{and} \quad (5)$$

$$\bar{a}_{max} = \frac{V_b}{R_m}. \quad (6)$$

(Below, we further divide \bar{a}_{max} by the time-zero mean phenotype to yield a measure of the proportional reduction in phenotype per homozygous mutation.) Formulae for the standard errors of these estimators were derived in Vassilieva and Lynch (1999):

$$SE(U_{min}) = U_{min}\{[2CV(R_m)]^2 + [CV(V_b)]^2\}^{1/2} \quad \text{and} \quad (7)$$

$$SE(\bar{a}_{max}) = \bar{a}_{max}\{[CV(R_m)]^2 + [CV(V_b)]^2\}^{1/2}, \quad (8)$$

where $CV(R_m)$ and $CV(V_b)$ are coefficients of sampling variation (ratio of standard error to estimated value) of R_m and V_b .

The Bateman-Mukai (BM) technique employs a method of moments that relies entirely on two properties of the distribution of line means, the mean and the variance among lines, and otherwise ignores the form of the distribution. Equations (5) and (6) are obtained under the assumption that mutations have constant effects; if this assumption is violated, U_{max} will be downwardly biased and \bar{a}_{max} will be upwardly biased. A

potentially less biased approach is a maximum-likelihood (ML) procedure that uses all of the information in the distribution of line means (Keightley 1994, 1996). This technique has limitations as well because, unlike the BM estimators, ML estimators make specific assumptions about the forms of both the mutational and environmental effects. In addition, the ML algorithms that are currently available use only the results from a single generation, whereas the BM estimators simultaneously employ the results from all assays. Nevertheless, insight into the robustness of conclusions derived from mutation-accumulation experiments can be obtained by comparing the results obtained by both methods.

The strategy of the ML approach is to evaluate the genomic mutation rate and distribution of mutational effects that best explain the departure of the distribution of mutation-accumulation line means from that of the nonmutated controls. We performed ML analysis using procedures similar to those introduced by Keightley (1994, 1996). As in previous applications of this approach, we assumed that the number of mutations per line was distributed in a Poisson fashion with expectation $Ut/2$, where U is the diploid genomic mutation rate and t is the number of generations of mutation accumulation, and we performed analyses under the assumption of either constant mutational effects or a gamma distribution of effects. However, our treatment of the residual errors of line means (caused by environmental effects) is a significant departure from the approach employed in previous studies.

Previous ML analyses of mutation-accumulation experiments have assumed that the residual errors of individual measures are normally distributed (Keightley 1994, 1996, 1998; Keightley and Caballero 1997; Keightley and Ohnishi 1998), but these assumptions are not justified with our data. Variation in the magnitude of residual errors of line means arises in our analyses for the simple reason that, because of loss of replicates during an assay, the sample size can vary from one to five individuals. More significantly, the distributions of residuals for the characters that we have analyzed are distinctly nonnormal, even when the means are based on five measures. We attempted a variety of transformations to achieve normality, including optimal Box-Cox transformations, but in all cases significant departures from normality remained. Thus, to describe the distribution of residual errors, we evaluated the deviations of individual measures from the control means standardized by the within-assay standard deviation, using the 638 measures that were available over seven assays (Fig. 1). The original empirical distributions were obtained by partitioning the individual measures into 50 discrete classes, and each of the observed frequency classes were then further subdivided fivefold by interpolation to yield a total distribution involving 250 classes. To obtain the distribution of residual deviations for line means involving each higher number of replicates ($n = 2, 3, 4,$ and 5), sets of replicates were drawn randomly from the original ($n = 1$) distribution to generate 10^6 line means, which were then allocated to the 250 classes. By the central limit theorem, one expects the distribution of residual errors of means to converge on normality as the sample size increases, but as can be seen in Figure 1, substantial nonnormality remains even at our largest sample size of five replicate individuals.

We used the time-zero intercept of the regressions of the

mutation-accumulation lines on time as the mean at time zero. The justification for this approach (as opposed to using the actual control mean) is the relative constancy of the control means over time (see below) and the slight elevation of the mutation-accumulation line means early in the experiment over the controls, apparently a consequence of a freezer effect (Vassilieva and Lynch 1999). The phenotypic standard deviation of the control individuals was taken to be the average of that observed over all assays.

For the ML analyses involving constant mutational effects, the expected distribution of mutation-accumulation line means involving n replicates, which is conditional on a specific mutation rate and effect, was obtained by modifying the control distribution to account for the expected shift due to mutations. To accomplish this, each phenotypic class in the control distribution was subdivided into all possible subclasses involving mutation number (using the Poisson distribution to determine the relevant frequencies), with each subclass being shifted in the downward direction by an amount equal to the product of the number of mutations and the homozygous mutational effect. For a given mutation rate and homozygous mutational effect, the log-likelihood of each observed mutation-accumulation line mean based on n replicates was obtained by simply taking the log of the expected frequency from the simulated distribution of expected line means given the sample size n .

The ML estimates of U and a were obtained by comparing the sums of the line-specific log-likelihoods obtained for different parameter estimates. Starting with a genomic mutation rate of 0.001 per generation, alternative mutation rates were examined in increments of 0.001, with each mutation rate being evaluated over an array of up to 1500 closely spaced mutational effects. Approximate 95% confidence intervals for the parameter estimates were obtained by identifying the combination of U and a that yielded the ML of the observed data and then extending the confidence interval in both directions from the ML estimate of U until the total log-likelihood dropped two units below the maximum.

Likelihood analyses involving variable effects proceeded in an identical manner as those for the constant-effects model with the additional need to account for the variation among lines with identical numbers of mutations resulting from the variable mutational effects. As in Keightley and Ohnishi (1998), we simplified the necessary computations by taking advantage of the fact that the sum of independent gamma-distributed variables is also gamma in form, with modified parameters (assuming that all mutational effects are members of the same gamma distribution). In the derivation of the expected empirical distributions of line means, for any particular number of mutations in a line, we generated 100 random sums of mutational effects, again convoluting the distribution of genetic effects with the expected distribution of residual effects given the sample size n and integrating this over the full Poisson distribution of mutation numbers. To test for the presence of significant variation of mutational effects, we again evaluated the log-likelihoods of the observed line means over the matrix of mutation rates and average effects noted above, with the addition of a third dimension—the coefficient of variation (ratio of the standard deviation to the mean) of mutational effects. Starting with a

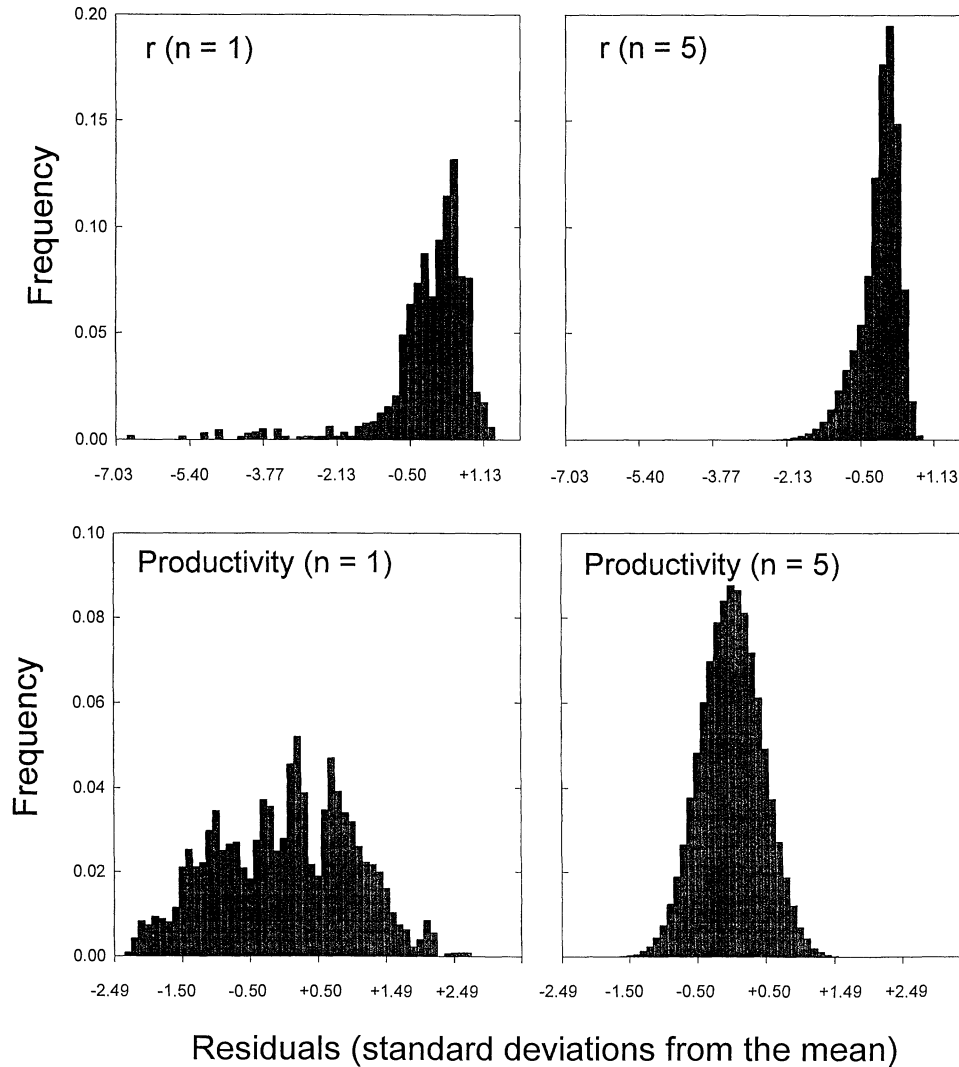


FIG. 1. Distribution of the residual errors for the intrinsic rate of increase, r , and productivity obtained from a collection of 638 control measures. The panels on the left are the distributions of standardized deviates of individual measures (deviation from the mean, divided by the within-line standard deviation), whereas those on the right are the expected distributions of residual errors for line means based on five replicates, obtained by randomly drawing individual deviates from the distributions on the left.

coefficient of variation of 0.0 (the constant-effects model), log-likelihoods were obtained at increasing values of the coefficient of variation in increments of 0.1. Variation of mutational effects was interpreted as being statistically significant if twice the difference between ML values under the variable-effects model and the constant-effects model exceeded the critical χ^2 -value with one degree of freedom.

Estimates of the Average Degree of Dominance

To estimate the average degree of dominance of mutations, we crossed random generation-170 mutation-accumulation lines to the control stock and evaluated the F_1 performance in a common environment including the parental mutation-accumulation lines. *Caenorhabditis elegans* has an X0 system of sex determination, with XX zygotes giving rise to hermaphrodites and X0 zygotes developing into males. Mild heat shock greatly elevates the frequency of nondisjunction of the

X chromosome during gametogenesis, resulting in approximately 2–5% male production (Hodgkin 1983, 1988).

To obtain males, three to four plates, each containing 10 young hermaphrodites from a line were exposed to mild heat shock at 26.5°C for 12 h, after which they were returned to 20°C for four to five days and examined for males. Males were then transferred to fresh dishes and crossed with hermaphrodites of the young adult stage from the same strain, typically in a ratio of 15 males to five hermaphrodites. The male-containing lines were then frozen using the protocol of Lewis and Fleming (1995). For four mutation-accumulation lines, we were unable to produce males in high enough densities to enable successful freezing. All four of these lines were visibly unhealthy, with one of the two sexes possessing an aberrant phenotype of the mating structures (either a protruded vulva in hermaphrodites or an abnormal tail morphology in males). In the work described below, we randomly

TABLE 1. Regressions for temporal changes in the means of the mutation-accumulation lines; r is the correlation coefficient. The percent change per generation is relative to the time-zero mean (slope/intercept). Standard errors are given in parentheses. The final column gives the ratio of the regression coefficients involving means obtained with and without the correction for selective line extinction.

Character	Intercept	Slope	r	% per generation	Correction factor
Productivity	200.095 (17.185)	-0.42737 (0.08102)	0.89	-0.214	1.26
Survival to maturity	0.982 (0.020)	-0.00057 (0.00010)	0.91	-0.058	1.73
Longevity	16.214 (0.731)	-0.00380 (0.00372)	0.38	-0.023	12.14
Intrinsic rate of increase	1.370 (0.083)	-0.00205 (0.00039)	0.89	-0.150	1.31
Rate of convergence	1.209 (0.085)	-0.00126 (0.00049)	0.77	-0.104	1.07
Generation rate	0.287 (0.003)	-0.00010 (0.00001)	0.94	-0.035	1.10

used 17 of the 72 stocks of frozen males. After thawing the lines, they were incubated at 20°C for two generations prior to the line-cross experiment to allow for recuperation from any physiological consequences of freezing.

Hermaphroditic *C. elegans* have a sperm:oocyte ratio of about 300:1000, and all sperm are normally used for self-fertilization over a span of three to six days (Lewis and Fleming 1995). For a few hours after a hermaphrodite runs out of sperm, unfertilized oocytes are expelled onto the plate. Such oocytes are relatively easy to distinguish from fertilized eggs based on shape and color, but in questionable cases, plates were stained with a 0.001% solution of trypan blue. Crosses were conducted by combining spent hermaphrodites and young males in an approximately 5:17 ratio. Further verification that the progeny were products of a cross was obtained by ascertaining that the sex ratio in progeny was 1:1. All crosses were made reciprocally in at least three to four replicates, and the control and mutation-line individuals represented in the final analysis were also products of within-line crosses. In three different blocks of crosses, all at 20°C, we obtained the phenotypic means of the reciprocal F_1 crosses in parallel with those of the parental mutation-accumulation lines.

For any mutation with homozygous effect a in a mutation-accumulation line, the effect will be altered to ha , where h is the coefficient of dominance, in the F_1 progeny of a cross to the control. Thus, for each block, the average degree of dominance (\bar{h}) was obtained by dividing the covariance between F_1 and parental mutation-accumulation line mean phenotypes by the genetic variance among the parental mutant lines, and an overall estimate of \bar{h} was obtained by averaging over the three blocks. An estimate of $\bar{h} = 0.5$ implies additive gene action, whereas $\bar{h} = 0.0$ implies that the mutant alleles are completely recessive.

RESULTS

Effects of Mutations Expressed in a Benign Environment

We first consider the results from assays performed under benign temperature conditions. Except for longevity, the mean phenotypes of all of the life-history characters expressed at 20°C declined significantly over the course of the experiment (Table 1, Fig. 2). Throughout the 214-generation period, the control means remained quite stable, except in the case of longevity and generation rate. Only in the case of these two traits was there significant covariance between the control fluctuations and the mutation-accumulation line

means, so except for longevity and generation rate, there appears to be no justification for correcting for temporal environmental trends. Relative to the estimated time-zero means, all of the mutation-induced changes in mean phenotypes were on the order of 0.02–0.21% per generation. Disregarding the aberrant estimate for longevity, these estimates are between 7% and 73% higher than those obtained if the correction for selective line extinction is not made (Table 1).

The among-line variance increased significantly with generation number for all of the characters in the mutation-accumulation lines, while there was no temporal trend in the controls for any trait except generation rate (Table 2, Fig. 3). Significant temporal covariance existed among the control and mutation-accumulation line means for longevity and productivity, and these were corrected as described above. Using half the rate of increase in the among-line variance as an estimate of the mutational variance and the average within-line variance as an estimate of the environmental variance, the mutational heritabilities for all of the characters fall in the fairly narrow range of 0.0008–0.0033 (Table 2).

As an example of the general temporal dynamics of the distribution of line means over the course of the experiment, some of the results for the intrinsic rate of increase, a composite measure of fitness incorporating viability and the age-specific schedule of progeny production, are illustrated in Figure 4. Here it can be seen that the distribution of line means gradually becomes increasingly skewed and broadened over time compared to the controls. The highest fitness classes observed in the controls are lost completely, whereas low fitness classes that are unobserved in the controls become relatively common.

The BM estimates of the genomic mutation rate for all characters fall in the range of 0.0005–0.0489, with an average value of 0.0168 (Table 3). If the statistically unreliable estimate for longevity is disregarded, the range narrows to 0.0030–0.0489 and the mean increases to 0.0200. The upwardly biased estimates of the average deleterious homozygous effects of mutations, scaled to the time-zero phenotypic means of the traits, range of 8–90% with an average value of 30%, and narrow to a range of 8–39%, with an average of 17%, if the estimate for longevity is excluded (Table 3).

We restricted our ML analyses to the two composite measures of fitness, r and productivity, both of which are easily interpreted biologically and exhibit relatively high stability of the control means over the course of the experiment. Under

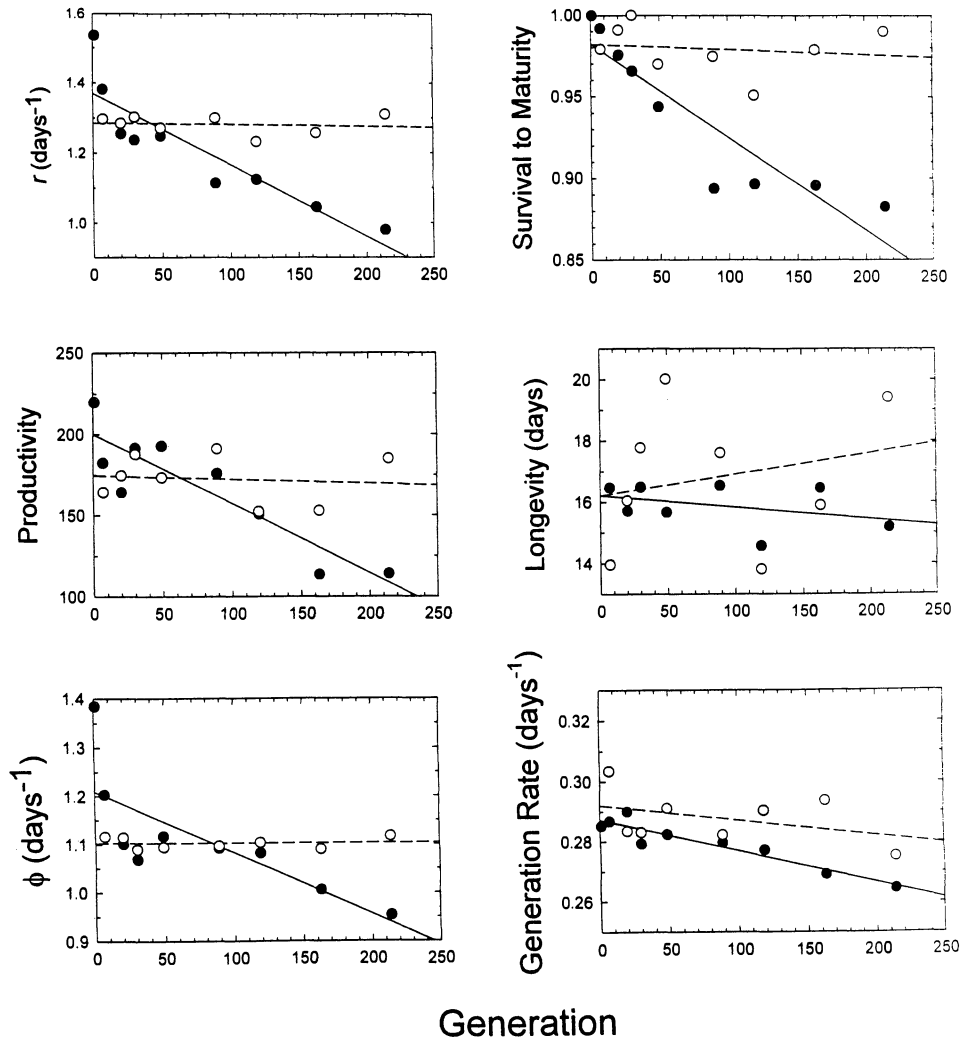


FIG. 2. Regressions of the mutation-accumulation line means (closed circles) and the control line means (open circles) on generation number. The mutation-accumulation line means are corrected for selection as described in the text, with those for longevity and generation rate being additionally corrected for temporal covariance with the control means.

the assumption of constant mutational effects, the ML method yields estimates of the genomic mutation rate that tend to increase with the generation number assayed (Table 4). In contrast, the estimated homozygous mutational effects decline with increasing assay number. The five point estimates for the genomic mutation rate for productivity range from 0.006 to 0.048, with no upper confidence limit exceeding 0.118 per genome per generation, whereas the estimates for the homozygous effect (proportional to the mean) range from

0.09 to 0.64. Averaging over the five ML estimates for this trait, the genomic mutation rate is estimated to be approximately 0.018, which is reasonably consistent with that obtained by the BM approach (0.049) considering the magnitude of the confidence intervals. In contrast, the average mutational effect (0.369) obtained by ML is fourfold higher, and apparently significantly so, than the BM estimate (0.088). For the intrinsic rate of increase, the entire range of the five estimated confidence intervals for U is 0.005 to 0.030, where-

TABLE 2. Regressions for temporal changes in the among-line variances of the mutation-accumulation lines; r is the correlation coefficient; h_m^2 is the mutational heritability. Standard errors are given in parentheses.

Character	Slope	r	h_m^2
Productivity	7.474584 (2.368799)	0.77	0.0008 (0.0003)
Survival to maturity	0.000218 (0.000029)	0.94	0.0031 (0.0007)
Longevity	0.055943 (0.013189)	0.87	0.0011 (0.0003)
Intrinsic rate of increase	0.000621 (0.000122)	0.89	0.0033 (0.0008)
Rate of convergence	0.000117 (0.000034)	0.79	0.0023 (0.0007)
Generation rate	0.000003 (0.000000)	0.91	0.0023 (0.0006)

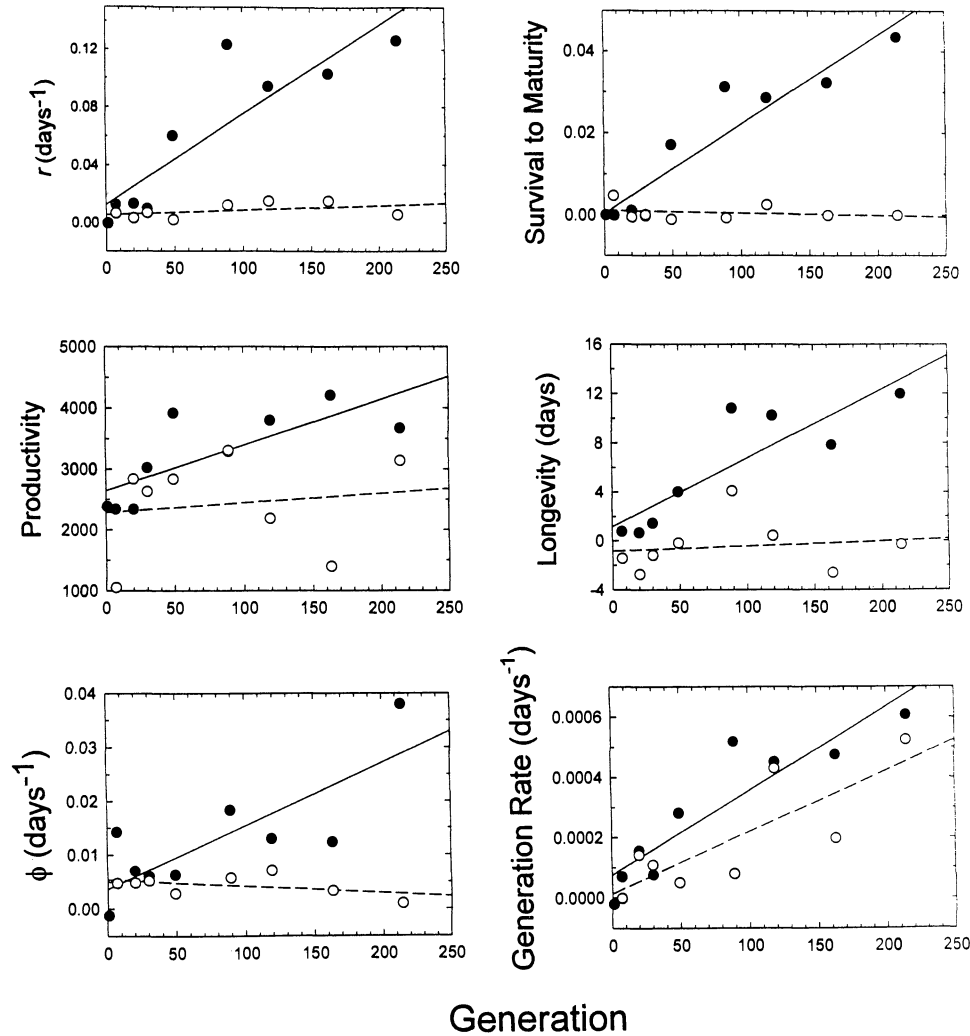


FIG. 3. Regressions of the among-line variance estimates for the mutation-accumulation lines (closed circles) and the controls (open circles) on average generation number. The mutation-accumulation line variances are corrected for selection as described in the text, and those for productivity and longevity are additionally corrected for temporal covariance with the estimates of among-line variance for the control lines.

as the point estimates fall in the narrow range of 0.010 to 0.018, and the point estimates for the homozygous effect fall in the range of 0.11 to 0.30. For this trait, the average estimate of U is identical to that obtained with the BM approach (0.014) and the estimated homozygous effect (0.20) is only slightly less than the BM estimate (0.22).

In all assays for productivity, the ML solution to the variable-effects model converged on the results of the constant-effects model, with the confidence intervals focused around the ML estimates for U also having associated coefficients of variation of mutational effects equal to zero (except in one assay where the coefficient of variation associated with the upper limit to U was equal to 0.5). In contrast, the incorporation of variable effects significantly improved the fit of the observed data for the intrinsic rate of increase in several assays, yielding higher estimates of the genomic mutation rate, lower estimates of the average effect, and coefficients of variation of effects in the range of 0.5 to 1.5 (Table 4). For this character, for some assays, a rather high genomic

mutation rate (as high as 0.35 in the final assay) and a rather low average homozygous effect (as low as 0.007 in the final assay) are consistent with the variable-effects model, but the upper confidence limit involving the coefficient of variation of mutational effects is never greater than 2.5.

Mutational Effects in a Stressful Environment

A reduction in the assay temperature from 20°C to 12°C resulted in a dramatic decline in the means of fitness-related characters—39% for productivity, 7% for viability to maturity, and 70% for the intrinsic rate of increase (Table 5). (The results for the 20°C assays reported in this section involve only those run in generations 163 and 214, the same as those for the assays at 12°C, thereby insuring that the comparisons involve the same lines.) The estimated rate of decline of the mean phenotype and the mutational coefficient of variation (ratio of the mutational variance to the mean phenotype at time zero) were higher for all characters ex-

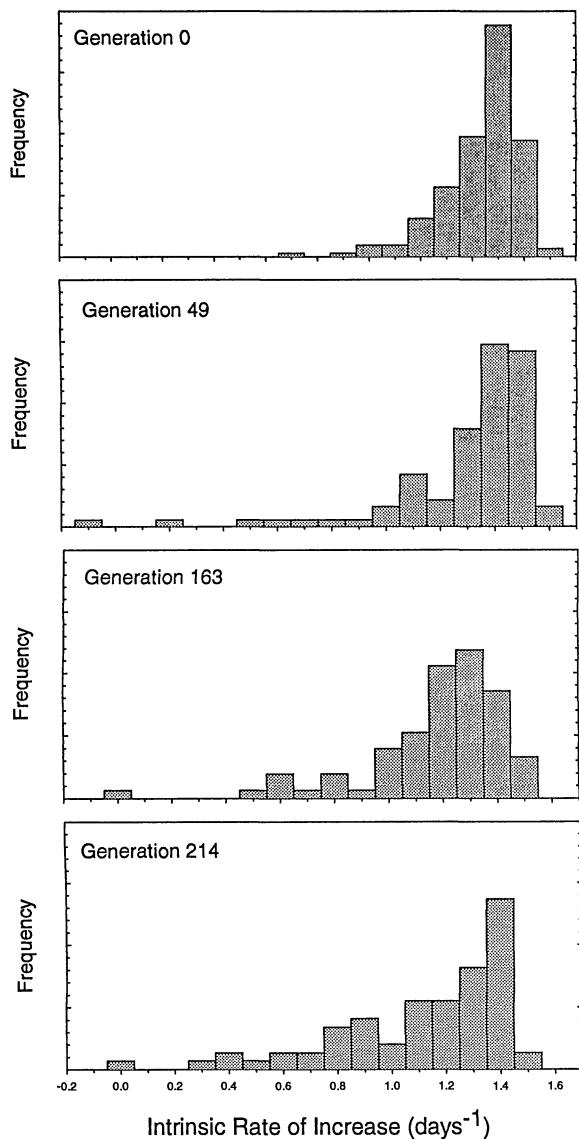


FIG. 4. The evolution of the distribution of line means for the intrinsic rate of increase over time. The control (time-zero) distribution summarizes the distribution of all control groups (each containing five replicates) over the entire course of the experiment.

pressed at 12°C than at 20°C (Fig. 5). These differences are highly significant for the composite measure of fitness (the intrinsic rate of increase), for which the mean expressed at 12°C declined at a rate of 0.15% per generation (nearly double the rate observed at 20°C) and for which the mutational coefficient of variation was 2.3% (more than five times that at 20°C).

These types of changes in the distribution of line means relative to the controls at low temperature may be a result of the expression of more deleterious mutations, a magnification of the effects of mutations, or a combination of the two. To gain some insight into this matter, we applied the BM method to the results at the two different temperatures. The results for 20°C reported here differ somewhat from those reported above, as they depend only on assays at generations 163 and 214, and no attempt has been made to correct for

bias resulting from selective line loss. Moreover, with only two assays involved, the power of this analysis is not particularly strong. Nevertheless, it can be seen that for all traits $U_{min,12}$ is equal to or lower than $U_{min,20}$, whereas $\bar{a}_{max,12}$ is equal to or greater than $\bar{a}_{max,20}$. Relative to their standard errors, the differences involving the intrinsic rate of increase are quite large. The genetic correlations across environments were strongly positive and highly significant ($P < 0.01$) in all cases (in generations 163 and 214, respectively, the point estimates for the intrinsic rate of increase are 0.44 and 0.73, for productivity are 1.34 and 1.65, and for survival to maturity are 0.30 and 0.69).

Estimates of the Average Degree of Dominance

The average degrees of dominance for the four composite parameters—intrinsic rate of increase, rate of convergence, generation rate, and productivity—are all statistically consistent with the hypothesis of additive effects of mutations ($\bar{h} = 0.5$; Table 3). However, the standard errors are relatively large, and the data are also compatible with values of \bar{h} as low as 0.10. In contrast, the point estimates for the average degree of dominance for mutations affecting viability and longevity are consistent with complete recessivity ($\bar{h} = 0$), although, again, because of the large standard errors of the estimates, partial recessivity with \bar{h} on the order of 0.3 to 0.4 cannot be ruled out.

DISCUSSION

This 214-generation study illustrates the statistical limitations of short-term mutation-accumulation experiments, particularly with organisms with low genomic mutation rates. In our previous 50-generation experiment (Vassilieva and Lynch 1999), the estimated rate of change in the mean phenotype was not statistically significant for most life-history traits, and Keightley and Caballero (1997) had the same experience with a similar 60-generation experiment. Our previous study actually suggested a slight (although nonsignificant) increase in productivity with mutation accumulation, and the rate of decline in longevity appeared to be significant. With increasing numbers of assay generations, a clearer picture has emerged. There is a highly significant decline in the mean for all characters except longevity with mutation accumulation, and the divergence of line means is highly significant for all traits. The estimates of mean longevity behave quite erratically for reasons that unfortunately remain unclear to us, but if the statistically unreliable estimate for this character is ignored, the rate of decline in the means for all fitness-related traits fall in the range of 0.04–0.21% per generation, with an average of 0.11% (0.03). The mutational heritabilities for all of the traits fall in the narrow range of 0.001–0.003, with an average value of 0.0021. The latter values are consistent with those obtained in many species (see reviews in Lynch and Walsh 1998; Lynch et al. 1999), but the former values are about an order of magnitude lower than early estimates from *Drosophila* mutation-accumulation experiments.

Using the BM technique and averaging over all traits except longevity, we infer that the genomic deleterious mutation rate for this species must be at least 0.02 per trait per gen-

TABLE 3. Bateman-Mukai estimates of the genomic mutation rate (U_{min}) and average homozygous effect (\bar{a}_{max}) of spontaneous mutations derived from the observed rates of change of the line means and variances, and the mean dominance coefficient (\bar{h}) obtained from backcrosses to the control.

Character	U_{min}	\bar{a}_{max}	\bar{h}
Productivity	0.0489 (0.0241)	-0.088 (0.032)	0.64 (0.18)
Survival to maturity	0.0030 (0.0011)	-0.390 (0.084)	0.05 (0.14)
Longevity	0.0005 (0.0010)	-0.908 (0.914)	-0.10 (0.28)
Intrinsic rate of increase	0.0135 (0.0058)	-0.222 (0.060)	0.55 (0.18)
Rate of convergence	0.0272 (0.0189)	-0.076 (0.032)	0.48 (0.19)
Generation rate	0.0077 (0.0024)	-0.094 (0.020)	0.69 (0.29)

eration. Although BM estimates of U are downwardly biased, some caution needs to be exercised in interpreting them as strict minimum possible values, because they are estimated with error. Nevertheless, additional estimates obtained by ML procedures appear to corroborate the idea that individual worms incur at least 0.02 new mutations affecting fitness per generation, particularly when one considers that these, unlike the BM estimates, do not account for selective line loss.

The average ML estimate of U for the intrinsic rate of increase under the variable-effects model, 0.024, is contained within the confidence interval of each of the five individual ML estimates and is also less than the upper confidence limit for the BM estimate. The degree to which BM estimates of U are downwardly biased is a function of the squared coefficient of variation of mutational effects, $C = \sigma_a^2/\bar{a}^2$, the estimate U_{min} being too low by a factor of $1 + C$. The average estimate of the coefficient of variation of mutational effects for the intrinsic rate of increase obtained by ML, 0.88, is contained within the confidence intervals for each of the five individual estimates, implying that $C \approx 0.77$ and raising our BM estimate of U for this trait from 0.014 to 0.024, the same as the average ML estimate. For productivity, the average ML estimate of U , 0.049, is more than twofold greater than the BM estimate, 0.018, although they are not significantly

different. For this character, ML analysis consistently suggested that $C \approx 0$, implying that the BM estimate is not downwardly biased. Thus, averaging over the BM and ML estimates for this trait, we conclude that U for productivity is approximately 0.033, perhaps slightly higher if the ML estimate is downwardly biased by the failure to factor in selection.

Again ignoring the aberrant estimate for longevity, all of our BM estimates for the average homozygous effects of mutations fall in the range of 8–39%, averaging to 17%. Some feeling for the robustness of this conclusion can again be acquired by considering the results obtained by ML analysis. The mean ML estimate for the intrinsic rate of increase, 13%, is contained within the confidence interval for the BM estimate of 22%, and these estimates become quite compatible when one corrects for the bias due to variance in mutational effects. Dividing the BM estimate by $1 + C$ yields a corrected estimate of 12%. Our conclusions on the average effects of mutations affecting productivity are somewhat more tentative. The ML analyses consistently failed to reject the constant-effects model, as was the case in the previous analysis of Keightley and Caballero (1997), so there is no justification for revising the BM estimate of 9% downwardly to account for variation in mutational effects. With an upper confidence

TABLE 4. Maximum-likelihood (ML) estimates of the diploid genomic mutation rate, U , and average homozygous effect (relative to the mean phenotype) \bar{a} , and for the case of the intrinsic rate of increase, the coefficient of variation of mutational effects, $CV(a)$. The estimates within parentheses define the confidence intervals associated with the likelihood profiles focused around the ML estimates of U . For the variable-effects model, χ^2 is twice the increase in the log-likelihood relative to that under the constant-effects model, and an asterisk indicates that the incorporation of variable effects into the model resulted in a significant improvement in fit ($P < 0.01$).

Generation	U	\bar{a}	$CV(a)$	χ^2
Productivity (constant-effects model):				
49	0.006 (0.003, 0.009)	-0.639 (0.687, 0.572)		
89	0.006 (0.003, 0.008)	-0.639 (0.696, 0.606)		
119	0.013 (0.007, 0.025)	-0.259 (0.367, 0.160)		
163	0.048 (0.030, 0.118)	-0.091 (0.141, 0.037)		
214	0.015 (0.008, 0.025)	-0.218 (0.340, 0.148)		
Intrinsic rate of increase (constant-effects model):				
49	0.010 (0.005, 0.015)	-0.295 (0.334, 0.286)		
89	0.011 (0.008, 0.015)	-0.292 (0.299, 0.292)		
119	0.012 (0.005, 0.016)	-0.170 (0.273, 0.164)		
163	0.018 (0.011, 0.030)	-0.111 (0.146, 0.063)		
214	0.018 (0.011, 0.022)	-0.109 (0.141, 0.103)		
Intrinsic rate of increase (variable-effects model):				
49	0.023 (0.007, 0.688)	-0.124 (0.229, 0.007)	1.5 (0.8, 2.5)	11.605*
89	0.014 (0.008, 0.049)	-0.233 (0.401, 0.080)	0.9 (0.7, 1.4)	10.713*
119	0.010 (0.005, 0.032)	-0.189 (0.262, 0.058)	0.5 (0.0, 1.0)	2.107
163	0.038 (0.023, 0.176)	-0.051 (0.076, 0.015)	0.6 (0.6, 0.9)	12.958*
214	0.036 (0.017, 0.350)	-0.058 (0.116, 0.007)	0.9 (0.7, 1.0)	11.285*

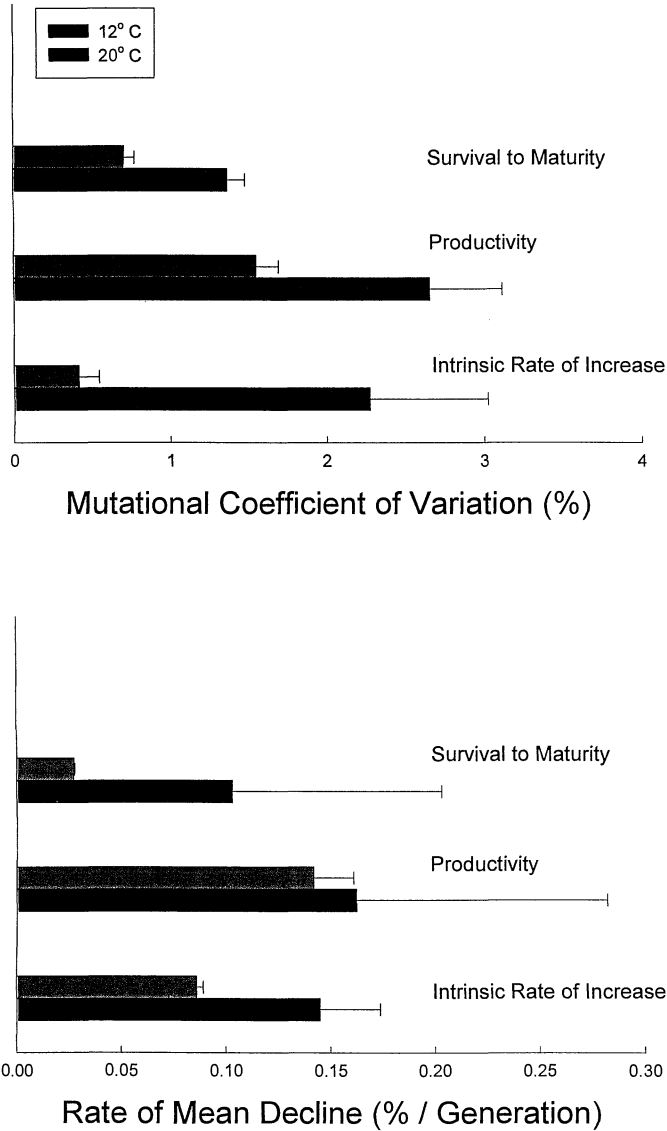


FIG. 5. Mutational coefficients of variation (the square root of the mutational variance divided by the mean phenotype) and the rate of decline of the mean phenotype (relative to the time-zero mean) for three fitness parameters, as expressed in a benign (20°C) and stressful (12°C) environment. Standard errors are denoted by the error bars.

limit of 15%, the BM estimate is then substantially lower than the average ML estimate of 37%, and below the statistical bounds of the earliest three of the five individual ML estimates. In contrast, the ML estimates declined dramatically with increasing number of generations of mutation accumulation, and the final two estimates are roughly compatible with the BM estimate.

The tendency for the ML approach to yield higher estimates of U and lower estimates of \bar{a} with increasing number of generations of mutation accumulation suggests that this method may simply be unable to detect small numbers of mutations with small effects in the early stages of a mutation-accumulation experiment with *C. elegans*. An alternative interpretation of the behavior of the ML analysis might be that

selective extinction of lines with mutations of large effects results in the differential survival of lines carrying only mutations with mildly deleterious effects late in our mutation-accumulation experiment. However, this explanation is inconsistent with the observed increase in estimates of U with increasing generation number.

Thus, when all considerations are taken into account, our data provisionally suggest that mutations affecting the intrinsic rate of increase in this species have a distribution close to exponential with a mean homozygous effect on the order of 12%, whereas the distribution of mutational effects on productivity has very low variance with an average effect similar to that for the intrinsic rate of increase. An average homozygous mutational effect on fitness of 12% is about half the previous estimates obtained for spontaneous mutations by Keightley and Caballero (1997) and Vassilieva and Lynch (1999) and for EMS-induced mutations by Davies et al. (1999). In contrast, our estimated average genomic deleterious mutation rate for fitness of approximately 0.03 is about sixfold higher than the previous estimate of Keightley and Caballero (1997) and about 50% greater than the previous estimate of Vassilieva and Lynch (1999).

Given the current state of uncertainty about the properties of spontaneous deleterious mutation in *Drosophila* (reviewed in Lynch et al. 1999), it is difficult to compare the results for this intensively studied species with those for *C. elegans*. However, a number of independent lines of evidence support the idea that U for total fitness in *Drosophila* must be at least 0.1 and perhaps as high as 1.0 (Lynch et al. 1999), recognizing that there is some disagreement on this (references provided in the introduction). If this interpretation is correct, then U for total fitness in *C. elegans* is on the order of 3–30% of that for flies, implying either that the genome of *C. elegans* is less mutable or that a smaller fraction of mutations influence fitness relative to the situation in flies.

We have previously suggested that a lower genomic mutation rate in *C. elegans* than in *D. melanogaster* may be a composite consequence of several biological differences between these organisms, including the substantially higher rate of transposition and the greater number of cell divisions per generation in *D. melanogaster* (Lynch et al. 1999; Vassilieva and Lynch 1999). The involvement of such scaling cannot be ruled out on the basis of the available data. The number of germ-line cell divisions in *D. melanogaster* is threefold higher than that in *C. elegans*, so if the mutation rate per generation scales with this trait, extrapolation from a *C. elegans* U of 0.03 yields a prediction of 0.09 for *D. melanogaster*. Accounting for the fact that approximately 40% of all mutations in *D. melanogaster* are due to the activity of transposable elements, which are quiescent in the N2 strain of *C. elegans*, extrapolates further to a predicted U of approximately 0.15 for *D. melanogaster*, which is compatible with the range of existing estimates for this species.

Most previous assays of *Drosophila* lines have been performed under competitive conditions, whereas the nematode assays of Keightley and Caballero (1997), Vassilieva and Lynch (1999), and those reported here have been performed at an optimal temperature with an unlimited amount of food. The results of our assays at low temperatures, far from the optimum for *C. elegans*, lead to the suggestion that on av-

TABLE 5. Mutation parameter estimates for fitness characters as expressed in harsh (12°C) versus benign (20°C) thermal environments. The mean phenotypes are given for the control lines. Standard errors are given in parentheses.

Trait	Temperature	Mean phenotype	U_{min}	\bar{a}_{max}
Intrinsic rate of increase	12	0.390 (0.019)	0.0041 (0.0037)	-0.709 (0.336)
	20	1.309 (0.030)	0.0449 (0.0356)	-0.038 (0.015)
Productivity	12	108.350 (20.853)	0.0039 (0.0060)	-0.838 (0.522)
	20	177.848 (7.043)	0.0095 (0.0048)	-0.301 (0.015)
Survival to maturity	12	0.912 (0.055)	0.0061 (0.0121)	-0.340 (0.326)
	20	0.981 (0.005)	0.0015 (0.0007)	-0.360 (0.013)

erage, U for fitness-related characters is reduced by a factor of 5.8 and \bar{a} is increased by a factor of 7.5 at 12°C relative to the situation at the more benign temperature of 20°C. These results suggest that although fewer mutations are expressed at stressful temperatures, the average mutational effect is inflated. However, it is possible that the effects of a few mutations are greatly exaggerated in harsh environments, overshadowing the effects of more numerous mutations of small effects. The strong positive genetic correlations across environments imply that a substantial fraction of the mutations incurred in these lines are expressed in both environments.

As in our study, Kondrashov and Houle (1994) and Shabalina et al. (1997) noted that the mean fitness of mutation-accumulation lines of *Drosophila* relative to controls declines more substantially under stressful conditions. However, contrary to our results, Fernández and López-Fanjul (1997) found no tendency for an increase in among-line variance with increasing stress and no evidence of a genetic correlation across environments. Unfortunately, joint information on the change in both the mean and the variance, necessary for the estimation of the contributions of mutation number versus average mutational effect in different environments, do not appear to be available in *Drosophila*. If our results with *C. elegans* are general, that is, if mutation-accumulation line assays in benign laboratory environments generally result in overestimates of U and underestimates of \bar{a} in harsh environments, then current estimates of the risk of extinction may be somewhat overly pessimistic in suggesting that small populations living in natural environments are likely to experience substantial accumulation of mutational load. Assuming that the natural environment is, in fact, more stressful than that encountered in laboratory conditions, because deleterious mutations of larger effects are more easily eliminated by natural selection, an increase in \bar{a} is expected to reduce the accumulation of mutation load at the population level. There is a clear need for further empirical investigation of the environmental dependence of mutational effects.

Finally, we consider our results on the dominance effects of new mutations. The magnitude of inbreeding depression, the probability of fixation of a new mutation, and the magnitude of genetic variance for fitness-related characters maintained under selection-mutation balance all depend on the degree to which the effects of newly arising deleterious mutations are masked in the heterozygous state. The available estimates of the average degree of dominance of new mildly deleterious mutations in *Drosophila* average to approximately 0.35 (Mukai et al. 1965; Mukai 1969), whereas estimates of \bar{h} for segregating deleterious alleles in natural populations of

flies and plants range from about 0.15 to 0.35 (Lynch and Walsh 1998, pp. 186–287). In other words, most of the existing data suggest that mildly deleterious mutations are partially recessive. Our results with *C. elegans* are consistent with this point of view. Although our estimates of \bar{h} for composite measures of fitness are not significantly different from the additive expectation ($\bar{h} = 0.5$), the standard errors of these estimates are large enough that \bar{h} as low as 0.1 cannot be ruled out. It should be emphasized, however, that our estimates of \bar{h} have been derived only for mutations expressed under benign conditions. Given the dramatic differences in the average effects of *C. elegans* mutations in harsh environments, it is conceivable that the degree of dominance is also environmentally dependent.

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