The Rate of Spontaneous Mutation for Life-History Traits in *Caenorhabditis elegans*

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ABSTRACT

Spontaneous mutations were accumulated in 100 replicate lines of *Caenorhabditis elegans* over a period of ~50 generations. Periodic assays of these lines and comparison to a frozen control suggest that the deleterious mutation rate for typical life-history characters in this species is at least 0.05 per diploid genome per generation, with the average mutational effect on the order of 14% or less in the homozygous state and the average mutational heritability ~0.0034. While the average mutation rate per character and the average mutational heritability for this species are somewhat lower than previous estimates for Drosophila, these differences can be reconciled to a large extent when the biological differences between these species are taken into consideration.

P RACTICAL interest in the properties of new mutations is motivated by two issues. First, the viability of species confined to a temporally changing environment ultimately depends on the rate at which mutation can generate new variation that can be assimilated into the adaptive evolutionary process (Lynch and Lande 1993; Lande 1995; Bürger and Lynch 1997). The rate of introduction of utilizable variation by mutation can also determine and ultimately limit the long-term rate of response of domesticated populations to artificial selection (Hill 1982; Falconer 1989; Falconer and Mackay 1996). Second, if the mean fitness of a population is to even remain constant in a stable environment, the recurrent introduction of unconditionally deleterious mutations must be balanced by their removal by natural selection. Small populations (especially those with restricted recombination) can be highly vulnerable to long-term mutational degradation if a significant fraction of mutations has mildly deleterious effects, *i.e.*, with effects less than the reciprocal of the effective population size (Lynch and Gabriel 1990; Lande 1994; Lynch *et al.* 1995a,b).

Inspired by these and other issues, a number of investigators have attempted to ascertain the mutational properties of quantitative traits. The data for a diversity of characters in an array of species suggest that mutation is generally sufficient to increase heritabilities by ~0.1–1.0% per generation (Lande 1975; Lynch 1988; Houle *et al.* 1996; Lynch and Walsh 1998). However, the extent to which this mutational variance is of actual

relevance to the adaptive evolutionary process is far from clear. Although the data are taxonomically limited, the existing evidence for Drosophila and *Escherichia coli* imply that the vast majority of mutations have deleterious effects on fitness (Simmons and Crow 1977; Crow and Simmons 1983; Crow 1993; Houle *et al.* 1996; Kibota and Lynch 1996; Lynch and Walsh 1998). If the effects of new deleterious mutations are unconditional, *i.e.*, disadvantageous in all ecological settings, then the observed high rates of mutational variance may provide a misleading perspective on the long-term evolutionary potential of species. On the other hand, a high rate of origin of deleterious mutations provides a potential explanation for a diversity of evolutionary genetic phenomena, including the ubiquity of inbreeding depression (Charlesworth and Charlesworth 1987; Lynch and Walsh 1998) and the evolution of senescence (Hamilton 1966; Rose 1991), recombination and sex (Muller 1964; Kondrashov 1988; Charlesworth 1990), and ploidy level (Kondrashov and Crow 1991).

To supplement the limited database on the fitness consequences of spontaneous mutations, we have initiated a long-term mutation-accumulation experiment with the nematode *Caenorhabditis elegans*, which normally reproduces by self-fertilization. The depth of understanding of the genetics, anatomy, and developmental biology of this organism probably exceeds that of any other metazoan (Riddle *et al.* 1997), and a number of other attributes make this species attractive for evolutionary genetic analysis: the simplicity with which lines can be maintained in the laboratory and in an inert frozen state, a short life cycle, and an ability to both self-fertilize and outcross. During the course of our investigation, another mutation-accumulation experiment with *C. elegans* was completed (Keightley and

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Caballero 1997), and we will compare and contrast the results of these two studies.

MATERIALS AND METHODS

Base strain and generation of lines: The mutation-accumulation experiment was initiated with a single individual derived from the wild-type Bristol-N2 strain, provided by the Caenorhabditis Genetics Center (St. Paul, MN). This strain had been maintained by inbreeding for dozens of generations before our experiment, and we further inbred a single lineage by self-fertilization for four generations before initiating our study with the selfed offspring from a single hermaphrodite. Thus, the amount of residual heterozygosity in the base individual should have been no more than expected under selection-mutation balance in a line containing a single individual. Such starting conditions provide an ideal setting for the analysis of a mutation-accumulation experiment (Lynch 1994). From the F_3 descendants of the base individual, 100 mutation-accumulation lines were initiated, while many thousands of additional animals were frozen and stored at $-80°$, as described by Lewis and Fleming (1995). This frozen stock of the original genotype served as a control for subsequent assays of the experimental lines.

Mutation accumulation: The nematodes were cultured and handled on NGM agar using standard techniques (Sulston and Hodgkin 1988) at $20°$. Individuals were maintained on 60×15-mm Petri dishes seeded with $\sim$80–90 μl suspension of *E. coli* strain OP50 (grown for 2 days at room temperature) 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 from the L3 to young adult stages. As a rule, to avoid selection for early or late reproduction (or accelerated or retarded development), we picked offspring that were produced in the middle of the parental reproductive period. To prevent accidental losses of the experimental lines, we always maintained two previous generations at $15°$ as potential backups. On average, $\sim$2% of the lines used backup individuals in each generation.

Assay procedure: The experimental lines were assayed with parallel controls on four occasions, at which times line divergence had proceeded for an average of 7.0, 19.9, 29.9, and 49.4 generations. Before each assay, each line was divided into replicates, all of which were transferred as single individuals for two generations, with single third-generation descendants from each replicate used in the actual assay. This procedure of line subdivision before analysis ensures that maternal and grandmaternal environmental effects do not contribute to the between-line component of variance, leaving it as a clean estimate of genetic divergence (Lynch 1985). The first assay contained five replicates from each of the experimental lines, while the second assay contained four replicates from 98 experimental lines, two lines having gone extinct. Assays 3 and 4 used five replicates from 98 and 95 lines, respectively, the slight reduction in line number, again, being a consequence of line extinction. 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. This resulted in 100 control individuals (five descendants from each of 20 thawed animals) being assayed in parallel with the mutation-accumulation lines. At the time of each assay, animals were transferred singly onto Petri dishes, using the youngest individuals in the L_1 stage, presumably within $\sim$3 hr of hatching, on the basis of their proximity to their eggshell and their size.

Estimation of life-history characters: *Productivity:* For the first assay, 48 hr $\pm$ 30 min after singly placing the L_1 individuals

onto Petri dishes, and every 24 hr $\pm$ 30 min thereafter, each individual was transferred to a fresh Petri dish with *E. coli* so that daily production of viable progeny could be recorded for the entire reproductive period. Because the first assay revealed that progeny production during the third day of life is negligible, for the remaining three assays, the first transfer took place at 72 hr $\pm$ 30 min, with subsequent transfers occurring 24 hr $\pm$ 30 min for only an additional 2 days. Results from the first assay demonstrated that 90% of total offspring production occurs within the first 4 days of reproduction, and we take the sum of viable progeny produced over this period to be a measure of total progeny production. To obtain this measure, after each daily transfer of the adult assay animals, plates with eggs were kept at $20°$ for a few additional hours to allow hatching. The plates were then stored at $4°$ until the progeny were counted. This treatment kills the larvae, and subsequent enumeration of progeny was facilitated by staining the agar pad and *E. coli* lawn with a 0.075% water solution of toluidine blue for 1.0–1.5 min, which leaves the dead worms transparent and visible on the contrasting purple background for the $\sim$5–10 min that are required for counting. Nonreproductive individuals were scored as having zero progeny production.

Survival to maturity: We scored an individual as having reached reproductive maturity if it produced at least one viable offspring.

Longevity: After the fourth day of life (during which individuals were examined daily), all worms were checked for survivorship every other day. An animal was considered dead only when pharynx contractions were not observed and there was no body or head reflex upon a gentle touch by a platinum wire.

Population growth parameters: The potential dynamic characteristics of worm population growth initiated by a single animal can be described in terms of the patterns of age-specific survival and fecundity following the logic presented in Svirezhev and Pasekov (1982) and Charlesworth (1994). We consider the situation in which: (1) survivorship to age x and fecundity at age x, $l(x)$, and $m(x)$, respectively, are age-dependent functions independent of absolute time; (2) the line is genetically homogeneous so that individuals can be classified solely by age; and (3) worms live in an unchanging environment without restriction by density-dependent factors. Under these assumptions, the expected population birth rate at time t resulting from a single initial ($t = 0$) individual can be represented by the function

$$B(t) = \sum_{j=1}^{\infty} C_j e^{z_j t},$$

where z_j are the roots of the characteristic equation

$$\int_0^{\infty} e^{-zx} l(x) m(x)\, dx = 1,$$

and C_j are constants determined by the initial conditions (Charlesworth 1994).

The largest real root, $z_1 = r$, is generally known as the intrinsic rate of natural increase, or the Malthusian parameter. As a composite measure of survival and reproductive output and timing, r provides a measure of exponential population growth for the situation in which a population has attained a stable age distribution and is expanding in a density-independent fashion. The rate of convergence of the population to the stable age distribution is largely a function of $z_2 = \phi + i(2\pi/T)$, which is defined as the root with second largest real value ϕ. The character z_2 determines the time it takes for a population to reach its exponential growth trajectory and, hence, the extent to which r by itself is a meaningful determinant of population dynamics (Svirezhev and Timofeeff-Ressovsky 1968). The relative contribution of this oscillatory

term to population dynamics can be significant during time periods of order $1/(r - \phi)$. The complex part of the second root, $i(2\pi/T)$, determines the frequency of oscillation in density for a population converging to its stable age distribution (Charlesworth 1994, pp. 40–41). The character T is defined to be the mean age of reproduction for a cohort of individuals, and $1/T$ can be viewed as the rate of turnover between generations. For the few individuals that never reproduced at all in our assays, we assumed an exponential decline of population size, setting $r = -1/t$, where t denotes the age of death; for these individuals, we treated ϕ and T as unobserved variables.

For each individual, the age-specific survivorship function was estimated by setting $l(x) = 1$ up to the age of mortality and $l(x) = 0$ thereafter. There are a number of potential measurement inaccuracies in the estimation of $m(x)$, that result from the observation at fixed times of a continuous egg-laying function, so we performed Monte Carlo simulations to obtain an approximate probability distribution of $m(x)$ for each worm and then used this to compute expected values of r, ϕ, and $1/T$ for each worm (appendix). These simulations also provided us with rough approximations of the contribution of measurement error to the within-line components of variance for these characters, which proved to be negligible.

Estimation of the genomic mutation rate and average effect: To obtain estimates of the diploid genomic mutation rate and the mutational effect, we used the method of Bateman (1959), and Mukai (1964), and Mukai *et al.* (1972). This indirect method of inference takes advantage of the fact that, in the absence of selection, the per-generation changes in the mean, R_m, and in the among-line variance of a quantitative trait for a set of mutation-accumulation lines, V_b, are functions of the diploid genomic mutation rate U, the average mutational effect $E(a)$, where $2a$ is the effect of a mutation in the homozygous state, and the average squared effect is $E(a^2)$. Provided that the regressions of the means and among-line variances on generation number are linear, their expected slopes are $UE(a)$ and $2UE(a^2)$, respectively. Downwardly biased estimates of the genomic mutation rate, U_{min}, and upwardly biased estimates of the average mutational effect, $\bar{a}_{max}$, are obtained by assuming $[E(a)]^2 = E(a^2)$ and solving

$$U_{min} = \frac{2(R_m)^2}{V_b},$$

$$\bar{a}_{max} = \frac{V_b}{2R_m}.$$

This approach can be applied to any character for which mutation causes a directional change in the mean.

We obtained estimates of R_m and V_b for each character by performing least-squares regression of the generation-specific means and among-line variances on the average generation number. The control means remained stable throughout the course of the experiment for all characters except longevity, which increased slightly as a consequence of our improved criteria for discriminating between live and dead worms. Thus, for this character, we estimated R_m by performing regressions with the deviation of the mutation-accumulation line means from the control means. The controls exhibited nonsignificant, among-line variance throughout the course of the experiment for all characters except total progeny production. For this character only, we estimated V_b by fitting the regression of the among-line variance for the mutation-accumulation lines with a nonzero intercept (which approximated the among-line variance for the control). For all other traits, we estimated V_b by fitting the regression through the origin.

Standard errors of the estimates of U_{min} and $\bar{a}_{max}$ were obtained by using the delta method to obtain expressions for the variance of a ratio (Equation A1.19b in Lynch and

Walsh 1998),

$$SE(U_{min}) = U_{min}\{[4CV(R_m)]^2 + [CV(V_b)]^2\}^{1/2},$$

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

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.

Estimation of mutational heritabilities: Under the assumptions outlined in the previous paragraph, half the rate of increase in the among-line variance ($V_b/2$) provides an estimate of the mutational variance $V_m = UE(a^2)$, which approximates the mutational rate of input of new genetic variance for a character with an additive genetic basis (Lynch 1994). We estimated the mutational heritability, *i.e.*, the ratio of mutational variance to the environmental component of variance (V_e), by use of the formula

$$h_m^2 = \frac{V_m}{V_e\{1 + [CV(V_e)]^2\}},$$

where $CV(V_e)$ is the coefficient of sampling variation of V_e. This expression accounts for the bias resulting from the sampling variance of the denominator of a ratio. The standard error of h_m^2 was estimated with the delta method expression

$$SE(h_m^2) = h_m^2\{[CV(V_m)]^2 + [CV(V_e)]^2\}^{1/2}.$$

RESULTS

Using two standard errors as a criterion for significance, all the traits exhibited highly significant mutational variance, with the mutational heritabilities falling in the narrow range of 0.0010–0.0056 and with an average value of 0.0034 (Table 1). Significant differences in mean phenotypes were observed for the experimental line means between the first and final assays for all characters except for productivity, while the control means were quite similar on both occasions (Figure 1). Based on regression analysis, relative to the initial means, the estimated per-generation rates of change in the mean phenotypes of all of the traits fell in the narrow range of 0.1–0.3%. The standard errors associated with the changes in mean productivity and in the population-dynamic parameters are relatively large, but the changes in survival to maturity and longevity, ∼0.1% and 0.07 days per generation, respectively, are highly significant. (Because we do not know the exact form of the distribution of means and variances, these statements about significance should be qualified somewhat. From Chebyshev's theorem, there is at least an 8/9 probability that a parametric value is within three SEs of the estimate, regardless of the form of the sampling distribution, so it is clear that there have been significant declines in survival to maturity and longevity in our lines, and the among-line variance remains significant for all characters.)

The distributions of mutation-accumulation line means at the first and final assays (generations 7 and 49; Figure 2) are reasonably informative, as the control line means were very similar on these two occasions (Figure 1). The frequency of individuals in the lowest

TABLE 1

Estimates of the initial mean ($\bar{z}_0$), rate of change of the mean (R_m), rate of input of mutational variance (V_m), environmental component of variance (V_e), and mutational heritability (h_m^2)

Character[a]	$\bar{z}_0$	R_m	V_m	V_e	h_m^2
e^r	3.94 (0.23)	−0.0071 (0.0073)	0.00263 (0.00079)	0.46862 (0.02739)	0.0056 (0.0017)
r	1.35 (0.06)	−0.0023 (0.0019)	0.00032 (0.00006)	0.06758 (0.00860)	0.0047 (0.0011)
ϕ	1.16 (0.06)	−0.0026 (0.0020)	0.00021 (0.00006)	0.04841 (0.00489)	0.0043 (0.0013)
$1/T$	0.30 (0.01)	−0.0654 (0.0428)	0.07918 (0.02585)	23.70000 (3.07630)	0.0033 (0.0012)
Productivity	170.75 (13.26)	0.4775 (0.4283)	9.74160 (2.97231)	4606.25000 (246.72089)	0.0021 (0.0007)
Survival	99.13 (0.59)	−0.0815 (0.0192)	0.54989 (0.18133)	206.95000 (48.49454)	0.0025 (0.0010)
Longevity	21.02 (0.65)	−0.0652 (0.0211)	0.03296 (0.00797)	31.48000 (2.84817)	0.0010 (0.0003)

[a] The traits r, ϕ, and $1/T$ have time units of days^{-1}, while productivity is measured in units of numbers of viable offspring, survivorship (to maturity) in percentage units, and longevity in units of days. Standard errors are given in parentheses. For $1/T$, $\bar{z}_0$ and R_m are multiplied by 100, and V_m and V_e are multiplied by 10,000.

classes for r, productivity, and longevity showed a clear increase over this period, and there was a distinct reduction in the incidence of individuals in the highest classes for r and longevity. Correlations between line means in the final assay suggest that the mutations arising in these lines have pleiotropic effects (Table 2). Such correlations are not strictly genetic correlations, as they contain a contribution from average environmental effects, but the fact that they are generally higher than those for the control lines (which deviate only because of environmental effects) suggests a strong genetic component.

Application of the Bateman-Mukai technique suggests that the minimum genomic mutation rate is in the range of 0.01–0.13 per trait per generation, the average estimate being 0.041 (Table 3). Relative to the time-zero mean phenotypes ($\bar{z}_0$ in Table 1), the upper-bound estimates of the average homozygous effects of the mutations are on the order of 5–24%, the average over all traits being 14%. These and the following analyses do not take into consideration the genetic events that might have been responsible for the extinction of five of our lines, none of which were lost as a consequence of negligence or laboratory accidents.

DISCUSSION

Focusing only on primary traits (*i.e.*, excluding the composite properties r, ϕ, and $1/T$), our estimates of the mutational heritability fall in the narrow range of 0.0010–0.0025, with an average of 0.0019. This average value is the lowest reported for any species for which multiple data sets are available. The average value for life-history characters in *Drosophila melanogaster*, 0.0033 (0.0009), derived from data summarized in Lynch and Walsh (1998) and from the recent studies of Fernández and López-Fanjul (1997), Pletcher *et al.* (1998), and Wayne and Mackay (1998) exceeds all of our point estimates for *C. elegans*.

Our average estimate of U_{min}, ~0.055 per diploid genome per generation for primary traits and 0.045 for all traits is also low compared to other estimates in the literature. For example, the results of several mutation-accumulation experiments give an average estimate of U_{min} of ~0.6 for both egg-to-adult viability in Drosophila (Crow and Simmons 1983; Lynch and Walsh 1998) and multiple life-history characters in Daphnia (Lynch *et al.* 1998). Indirect estimates of the genomic mutation rate for total fitness, obtained by comparing the attributes of selfed and outcrossed progeny, range from ~0.2 to 1.5 (B. Charlesworth *et al.* 1990; D. Charlesworth *et al.* 1994; Johnston and Schoen 1995), and more circumstantial evidence suggests that zygotic mutation rates for quantitative traits are commonly at least 0.2 per character per generation in eukaryotic species (Lynch and Walsh 1998, Chap. 12).

With an average value over all traits of 0.14, our estimates of the average homozygous effect $2\bar{a}_{max}$ (scaled to the initial mean of the character) are somewhat higher than the average estimate for egg-to-adult viability obtained from mutation-accumulation experiments with *D. melanogaster* ($2\bar{a}_{max} \simeq 0.06$; Crow and Simmons 1983). Upper-bound estimates of average heterozygous effects are on the order of 2% for life-history traits in Daphnia (Lynch *et al.* 1998), and the average effect of a spontaneous mutation on cell division rate in *E. coli* is ~1% (Kibota and Lynch 1996). Because the fitness effects of most mutations are partially recessive (Crow and Simmons 1983; Houle *et al.* 1997), these results collectively support the idea that the vast majority of mutations have relatively small deleterious effects on fitness, on the order of 5% or less in the heterozygous state.

As noted above, estimates of U_{min} for viability obtained by mutation-accumulation experiments in male Drosophila average to ~0.60 per diploid genome per generation, which is substantially higher than our observed average value of 0.055 for life-history traits in *C. elegans*. These differences can be reconciled by accounting for some of the biological differences between these two species. First, ~38% of all new mutations in *D. melanogas-*
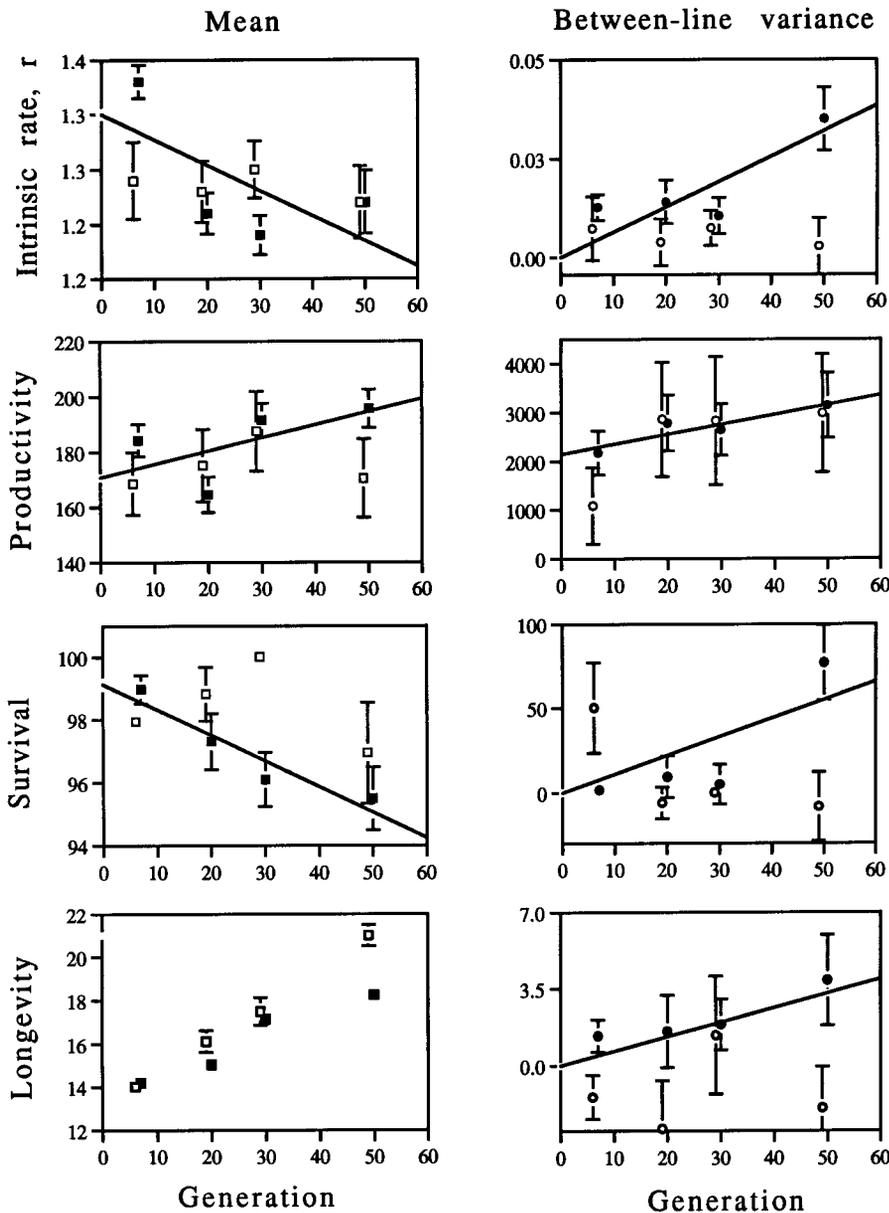
Figure 1.—Temporal changes in the means and among-line variances for several traits. Control values are plotted as open symbols, and values for mutation-accumulation lines are plotted as closed symbols. Vertical bars denote single standard errors; where they are missing, the standard errors are smaller than the width of the point. Before analysis, the data on the means for longevity were corrected for the changes in the control lines, as noted in the text, so this regression is not shown. The slopes of the regressions are equivalent to R_m and $2V_m$, as reported in Table 1. Note that for longevity, only the raw data are shown; as noted in the text, the regressions for the mean in this case were corrected for the temporal trend in the control.

ter appear to be a consequence of transposable element activity (Nuzhdin *et al.* 1995), whereas the strain of *C. elegans* with which we worked has no observable activity of mobile elements (Eide and Anderson 1985). Second, the number of cell divisions in the germ line of *D. melanogaster* is approximately four times larger than that in *C. elegans* (nine, on a per-generation basis, Hodgkin 1988; Kimble and Ward 1988; Wilkins 1993), and the generation time of the former is about one-fifth of that of the latter. Using the average value of the latter two differences (0.225) as a time-scale effect, the Drosophila estimate of U_{min} adjusts downwardly to 0.62 × 0.225 × 0.60 = 0.084, thereby reducing the 30-fold difference in U_{min} between worms in flies to a less than 2-fold difference.

If this difference is real and not just an artifact of sampling error, there are at least two potential biological explanations. First, the fly genome is a somewhat larger mutational target for fitness than the worm genome. The total genome size of Drosophila (in base pairs) is approximately twice that of *C. elegans* (Ashburner 1989; Hodgkin *et al.* 1995). Although the amount of coding DNA in the two species is similar, it is conceivable that the amount of noncoding regulatory DNA relevant to fitness in flies is higher than in worms, which are less complex developmentally. Second, some would argue that the higher estimates of U_{min} in Drosophila are a consequence of assays being performed on individuals in a competitive environment in which a greater fraction of the mutational load is expressed (Shabalina *et al.* 1997).

Although data on single-locus mutation rates are scant, those that exist provide further support for the idea that there is a reduction in the mutation rate in
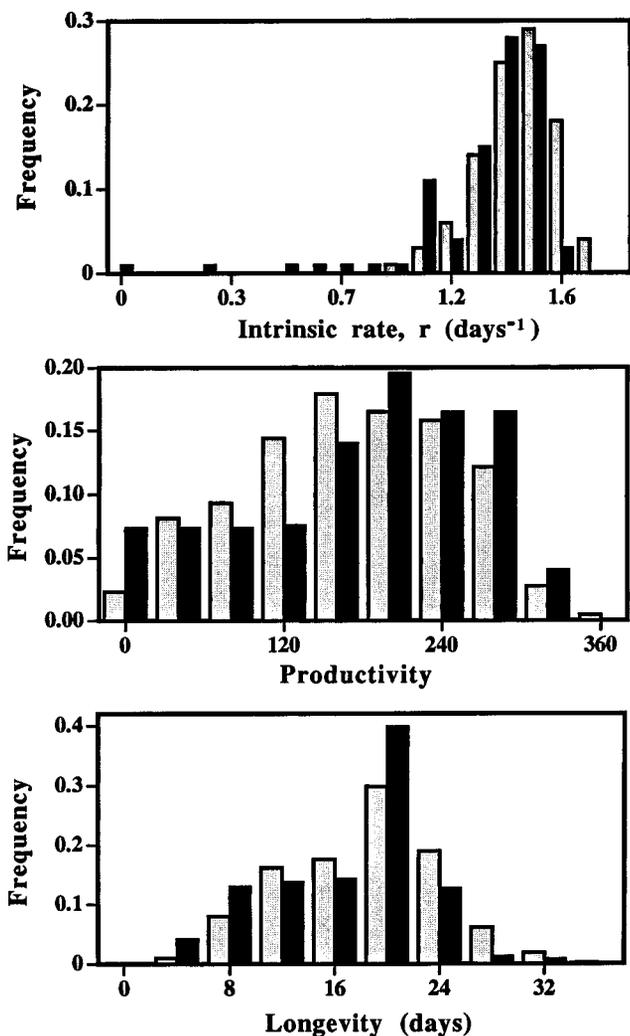
Figure 2.—The distribution of line means at generations 7 (gray) and 49 (black). The phenotypic values for longevity were corrected for the changes in the controls, as noted in the text.

of data taken from disparate sources yield results for flies and nematodes that are qualitatively consistent once the unique aspects of the biology of these two organisms are taken into consideration. This conclusion is inconsistent with recent arguments that the results from previous mutation-accumulation experiments with Drosophila are substantially flawed (Garcia-Dorado 1997), arguments that have been encouraged in part by the observation of low genomic mutation rates in *C. elegans* (Keightley and Caballero 1997). Other circumstantial evidence supports the validity of the Drosophila data. For example, the estimates of $U_{\min}$ from the Drosophila mutation experiments yield predictions about the expected inbreeding depression for fitness in natural populations that are reasonably compatible with observations in several species of Drosophila (Lynch *et al.* 1995a), although the suggestion has been made that the observed $U_{\min}$ may be too low unless there is significant synergistic epistasis (Charlesworth 1998). While one could argue that the inbreeding load in natural populations is maintained by overdominance rather than by recurrent mutation to deleterious recessives, this is inconsistent with the observation that the mean persistence times of mutations for fitness are on the order of several dozen generations (Crow 1992; Houle *et al.* 1996). Thus, we feel it is premature to reject the classical interpretation of the Drosophila data, a point that has also been made by Keightley (1996).

Recently, Keightley and Caballero (1997) reported the results of a 60-generation mutation-accumulation experiment (denoted as KC hereafter) applied to a descendant of the same strain used in our study. For total progeny production (which, as in our study, includes the egg viability component), they obtained an estimate of mutational heritability equal to 0.0012 (0.0008), which is not significantly different from our estimate of 0.0025 (0.0011). As in our study, KC did not observe a significant change in the mean productivity, although their observed change in the mean was slightly negative, while ours was slightly positive. The KC estimate of $U_{\min}$ of 0.0013 obtained by Bateman-Mukai estimation (and their maximum-likelihood point estimate of 0.0052) appears at face value to be substantially smaller than our estimate of 0.0234, but in light of the nonsignificant changes in the mean for this trait, this difference should not be taken too seriously. Taken together, both sets of results suggest that spontaneous mutations in *C. elegans* primarily expand the variance of total offspring production while having very minor effects on the mean. The absence of negative directional effects of mutations on fecundity is not unique to *C. elegans.* A slow increase in fecundity in a set of mutation-accumulation lines of Daphnia, which is coincident with a decline in other fitness-related characters (including viability, body size, and development rate), has recently been observed (Lynch *et al.* 1998), and similar observa-

C. elegans relative to that in *D. melanogaster* on a per-generation basis. The average rate of visible mutations per locus is $\sim 5 \times 10^{-6}$ per locus per generation in *D. melanogaster* (Crow and Simmons 1983), but only $\sim 7 \times 10^{-7}$ per locus per generation in *C. elegans* (Hodgkin 1974; Greenwald and Horvitz 1980; Eide and Anderson 1985). The average genomic rate of mutation to lethals is ~ 0.02 in *D. melanogaster* (Crow and Simmons 1983) and ~ 0.01 in *C. elegans* (Rosenbluth *et al.* 1983). Using purely molecular data, Drake *et al.* (1998) have recently estimated the minimum *total* mutation rate per effective haploid genome (coding DNA) in *C. elegans* to be ~ 0.004 per cell division (their estimate for Drosophila was 0.005). This estimate is remarkably similar to the minimum deleterious mutation rate per total haploid genome that can be derived from our data—$0.055/2/9 = 0.003$ per cell division.

The preceding computations should only be viewed as approximations, the main point being that a diversity

TABLE 2

Correlations among line means in generation 49

Character[a]	e^r	r	ϕ	$1/T$	Productivity	Survival	Longevity
e^r	—	0.98**	0.96**	0.78**	0.58*	0.29	0.55*
r	0.98**	—	0.98**	0.86**	0.49*	0.41	0.56*
ϕ	0.98**	0.99**	—	0.87**	0.42*	0.40	0.54*
$1/T$	0.91**	0.96**	0.96**	—	0.00	0.42	0.54*
Productivity	0.89**	0.82**	0.80**	0.64**	—	0.11	0.22
Survival	0.94**	0.92**	0.91**	0.94**	0.62**	—	0.22
Longevity	0.49**	0.54**	0.55**	0.48**	0.45**	0.58*	—

[a] The numbers below the diagonal refer to the 89 surviving experimental lines, whereas those above the diagonal are based on 20 control lines. One and two asterisks denote significance at the 0.05 and 0.01 levels, respectively.

tions have been made for *D. melanogaster* (Fernández and López-Fanjul 1996).

It is possible that total progeny production in *C. elegans* is normally under stabilizing selection, with an intermediate value for this trait leading to maximum total fitness (for example, as a consequence of negative pleiotropic effects of high fecundity on other fitness-related characters). If that were the case, then most of the mutations that we observed for this trait would be deleterious from the standpoint of total fitness, even though the Bateman-Mukai approach yielded a nonsignificant U_{min} (because of the nonsignificant change in mean productivity). There are a number of obvious pleiotropic interactions between the life-history traits of this species, and we simply provide one example. In the hermaphroditic stage of *C. elegans*, gametogenesis is normally a two-step process that initiates with spermatogenesis and then switches to oogenesis as a number of genes are activated. Hermaphroditic progeny production is limited by the number of sperm. Prolonged spermatogenesis of mutants increases total hermaphroditic fecundity, but it also leads to postponement of reproduction (Hodgkin and Barnes 1991). On the other hand, a reduction in spermatogenesis leads to a reduction in hermaphroditic fecundity. Future work should reveal whether this or other internal constraints underlie the

relatively stable mean levels of progeny production (but increasing among-line variance) that KC and we observed.

For the one other character shared between these two studies, longevity, KC estimated the mutational heritability to be 0.0004 (0.0004), which is lower but not greatly different from our estimate of 0.0010 (0.0003). Whereas we observed a significant decline of mean longevity, 0.066 (0.021) days per generation of mutation accumulation, KC found no significant change. Using a maximum-likelihood procedure, they obtained a minimum estimate of the genomic mutation rate for longevity (0.003) that is $\sim$43 times lower than our downwardly biased estimate (0.129). These two estimates are not strictly comparable, however, as the former is derived from a point estimate minus the sampling error, which is in principle unbiased if the specific model assumptions about the form of the distribution of mutational effects are fulfilled, whereas the latter is a downwardly biased estimate that uses a model that makes no assumptions about the form of the distribution of effects, but is not further diminished to account for sampling error. The most appropriate comparison between the two studies is the one that uses the same Bateman-Mukai estimator. For the KC data, this yields an estimate of U_{min} = 0.060 (P. D. Keightley and A. Caballero, personal communication) that is reasonably compatible with, although slightly lower than, ours.

In summary, for productivity and longevity, we obtained estimates of the mutational heritability that are about twice those of KC, and our estimates of U_{min} are also about twice as high. Although both our assays and those of KC were performed at identical temperatures and under similar feeding regimes, and both experiments had similar magnitudes of line extinction (5% line loss over 50 generations in our study, and 4% loss over 60 generations in KC), there are some differences in the protocols for line maintenance and for assays, which might be partially responsible for the differences in results.

First, all aspects of our work were conducted at 20°

TABLE 3

Estimates of the genomic mutation rate (U_{min}) and the average homozygous effect ($2\bar{a}_{max}$) scaled relative to the original mean

Character	U_{min}	$2\bar{a}_{max}$
e^r	0.019 (0.040)	−0.190 (0.204)
r	0.016 (0.027)	−0.210 (0.178)
ϕ	0.032 (0.050)	−0.138 (0.113)
$1/T$	0.054 (0.073)	−0.082 (0.060)
Productivity	0.023 (0.042)	0.240 (0.227)
Survival to maturity	0.012 (0.007)	−0.136 (0.055)
Longevity	0.129 (0.089)	−0.048 (0.019)

(except that our backup plates were kept at 15°), while KC maintained all their worms at 25° during the mutation-accumulation phase of their experiment. In *C. elegans*, hundreds of mutations are known to be temperature sensitive, yielding more severe phenotypes at 25° than at 20° (Guo *et al.* 1991; Sibley *et al.* 1996; Hodgkin 1997). Some mutations are lethal at 25° but viable at 20° (McKim *et al.* 1992). So it is possible that increased selection pressure against newly arisen mutations at higher temperatures is responsible for the lower rate of deleterious mutation accumulation in the experimental lines of KC.

A second feature of the KC experiment that may have led to downwardly biased rates of deleterious mutation accumulation is connected with the feeding regime and the maintenance of backups for line replacement. In the KC experiment, the food source for the worms was a 25-μl suspension of *E. coli* that was seeded as a spot and allowed to grow overnight, whereas our study routinely supplied 80–90 μl of the same type of suspension that was allowed to grow for 2 days. The amount of food used by KC is enough for one generation, but may be insufficient for the next one, in which case, strong competition might occur between animals on backup plates. In general, two generations of worms on one plate could constitute as many as 90,000 (300 × 300) individuals, which could result in significant starvation by the time backup individuals were actually used in the KC experiment (although no obvious starvation was apparent, P. D. Keightley and A. Caballero, personal communication). In our study, backup individuals were maintained at 15° and, hence, population growth rate was much lower. Moreover, an average of only 2% of our experimental individuals failed to reproduce during the course of our experiment, whereas KC report that ~7% of their worms failed to reproduce. Thus, if enhanced competitive conditions in the backup plates of KC magnified the intensity of selection against mutations, then the higher reliance on backup individuals in the KC study may have somewhat diminished the rate of mutation accumulation.

Finally, we note that some differences may have existed in the environmental conditions during the assay phases of the two experiments. Productivity in our control lines was on the order of 175 per individual (although, as noted above, we did not include the fraction of progeny produced late in life), whereas that for the KC lines was ~250. We have found that the total productivity of our control lines increases to ~220 after several generations removed from the freezer, but this does not entirely eliminate the difference between our observed average productivity and that of KC. On the other hand, the average longevity of our individuals was somewhat higher than that of the KC lines, 17 *vs.* 14 days. Thus, relative to the setting in our experiments, the conditions in the KC experiment appeared to promote higher fecundity but lower survivorship. We cannot, however,

rule out the possibility that these differences are caused by evolutionary changes in the Bristol-N2 strain before its analysis in one or both labs rather than by differences in husbandry procedures.

Despite these subtle differences, the results from both our study and that of KC lead to qualitatively consistent conclusions. Relative to *D. melanogaster*, *C. elegans* (at least the one strain that has been subject to study) has lower mutational heritabilities for life-history traits on a per-generation basis. Assuming the distributions of mutational effects in these two organisms are approximately the same, *C. elegans* also appears to have a substantially lower genomic rate of mutation per generation. The actual rates of mutation in both species may actually be substantially greater than the Bateman-Mukai estimates would suggest. With a variable distribution of mutational effects, U_{min} provides an estimate of $U/(1 + C)$, where U is the actual rate of genomic mutation, and C is the squared coefficient of variation of mutational effects (ratio of the variance of effects to the squared mean effect). Some evidence suggests that the distribution of mutational effects on fitness in Drosophila is highly leptokurtic (strongly L shaped), with C being substantially greater than one (Keightley 1994). If this is generally true, then the actual genomic mutation rate for life-history characters in *C. elegans* could easily be in excess of 0.1 per generation. In contrast, the actual average effect of a mutation is upwardly biased by the factor $(1 + C)$, so our results continue to support the idea that most mutations that affect fitness have effects smaller than a few percent in the heterozygous state.

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APPENDIX

When the precise reproductive function $k(x) = l(x) m(x)$ for animals of each age x is known, the problem of finding the maximum real value roots z_1 (or r), and z_2 (or $\phi + i2\pi/T$) of the characteristic equation

$$1 = \int_0^\infty k(x) e^{-zx} dx \qquad (A1)$$

is purely algebraic. In practice, however, we do not know the reproductive function with absolute accuracy. Instead, $k(x)$ is estimated from cumulative measurements of the number of viable progeny, $\hat{N}_j$, produced by adults during the fixed age intervals $(\hat{x}_{j-1}, \hat{x}_j)$,

$$\hat{N}_j = \int_{\hat{x}_{j-1}}^{\hat{x}_j} m(x) \, dx, \qquad (A2)$$

with our specific progeny counts being made at ages $\hat{x}_j$ of the fourth, fifth, sixth, and seventh days after hatch. In our experiment, we estimated the true reproduction function $k(x)$ by the stepwise approximation

$$\hat{m}_j = \frac{\hat{N}_j}{\hat{x}_j - \hat{x}_{j-1}}. \qquad (A3)$$

What impact does such simplification have on the determination of the fitness-related parameters r, ϕ, and $1/T$, and our ability to detect small changes in these caused by mutation accumulation? In addition to the fact that the approximation given by Equation A2 is not continuous, all the parameters contained within it clearly have some experimental uncertainties. The number of viable eggs, $\hat{N}_j$, could be counted incorrectly, and the age interval $(\hat{x}_{j-1}, \hat{x}_j)$ is not known exactly. We have denoted this randomness of the estimates with a ^ placed above them. Here we show that variation in the determination of fitness-related parameters associated with statistical uncertainties are negligible with respect to the typical within- and between-line variances.

The two primary sources of error in our inferences about $k(x)$ are the errors connected with counts of progeny on Petri dishes and the estimated ages of the parental hermaphrodite over which the progeny were pro-

duced. We performed Monte Carlo simulations of both sources of error in an attempt to establish an upper limit to the additional variance that might be associated with them. The analysis was conducted for each worm by generating 1000 solutions ($\hat{r}$, $\hat{\phi}$, $1/\hat{T}$) of Equation A1 with reproduction functions defined by Equation A3 obtained by sampling $\hat{N}_j$ and $\hat{x}_j$ values from the distributions described below. The mean values of these replicate parameter estimates were taken to be the final estimates for each individual, and the within-individual variance estimates were used to obtain limits to the variance caused by experimental error. In the following, we analyze each of two error sources in further detail to fulfill this approach.

Progeny counts: To approximate the $\tilde{n}$ distribution, we first experimentally estimated the variance in the estimates of offspring numbers that result from counting error. Because the estimated variance of counts was always less than the mean count, we concluded that if the real conditional probability of obtaining a certain count value, given a known n, is approximated with a Poisson distribution

$$P(\hat{N}|n) = e^{-n} \frac{n^{\hat{N}}}{\hat{N}!}, \qquad (A4)$$

the variance of counts will be overestimated. Thus, we assume that the Poisson distribution is an acceptable description for the goal of our investigation. If so, the conditional probability that the value of the actual number of progeny is contained within the interval of n to $n + dn$, given the observed value $\hat{N}$, satisfies the gamma distribution

$$P(n|\hat{N}) = e^{-n} \frac{n^{\hat{N}}}{\hat{N}!} \qquad (A5)$$

with mean and variance $\hat{N}$. This distribution was used in our Monte Carlo simulations to sample the actual number of produced eggs $\hat{n}_j$ based on the observed counts $\hat{N}_j$.

Age intervals: We chose the time of an egg's fertilization as the zero age moment ($x = 0$) in the life cycle. In general, it is irrelevant which moment in time should be considered as the zero age point (fertilization or hatch), and, because the reproduction dynamic of a worm is usually viewed as an egg-laying process, this is the customary approach. In addition, a number of mutations in *C. elegans* (such as *egl* mutations and some *unc* mutations) cause the eggs to hatch inside the uterus, making the estimation of hatch time troublesome.

To determine the age intervals of egg laying, one needs to know the amount of time passed from the moment when a parental zygote was produced to the moment of progeny egg fertilization, x_j. There are several contributions to the age $\hat{x}_j$ (in hours):

$$\hat{x}_j = x_{emb} + \hat{x}_{L_1} + 48 + 24j - 3 - \hat{x}_{add}. \qquad (A6)$$

The first term denotes the duration of embryogenesis. It is highly stable and equal to ~18 ± 1 hr for the temperature used in our experiment. The next term is the age of L_1 individuals used in an assay. Although we tried to pick the youngest L_1 larvae (see materials and methods), this term brings this fourth one of the major age uncertainties. We took the average age of L_1 individuals to be ~3 hr, and we took its maximum value to be the duration of the L_1 stage at the experimental temperature, *i.e.*, by 15 hr. After $48 + 24j$ hr, where $j = 1, 2, 3$, we routinely transferred the worms to fresh Petri dishes. The error of this time period is determined by our experimental design and is equal to ±30 min, which can be neglected on the background of error for the L_1 age. To estimate the reproductive function of a hermaphrodite, we counted larvae that hatched from eggs fertilized some time ago. Therefore, the next two terms occurred because of the uncertainties concerning the duration that the fertilized egg is in the uterus; in wild-type animals, usually it is ~3 hr, but the time can increase with the age of the hermaphrodite (Wood 1988). This additional time during which the fertilized eggs could be still in the uterus is described by the last term, $\hat{x}_{add}$, with a maximum limit of 15 hr at 20°, after which the embryos hatch.

Thus, to account for errors in the age intervals $\hat{x}_j$, we simulated two major sources of uncertainties $\hat{x}_{L_1}$ and $\hat{x}_{add}$. We used exponential distributions

$$f(x) = \frac{e^{-\alpha x/X_{max}}}{1 - e^{-\alpha}} \cdot \frac{\alpha}{X_{max}}, \quad 0 \leq x \leq X_{max}, \qquad (A7)$$

to describe these effects. Such distributions seem to be the most natural choice by the following arguments. First, we can make reasonable assumptions about only two parameters of real distributions—the mean values of $\hat{x}_{L_1}$ and $\hat{x}_{add}$ and their maximum limits X_{max}. Therefore, our estimates of the distributions must be two-parameter functions. Second, we expect them to be monotonic functions that rapidly decrease with increasing age.

Third, we want these distributions to have overestimated variances with respect to the real ones because our task is obtaining the upper limits of errors associated with the design of the experiment.

It can be shown that the form of the distribution given by Equation A7 can be reconstructed from the first and third arguments if one maximizes the entropy function $-\int_0^{X_{max}} f(x) \ln[f(x)] dx$, a condition very closely related to the maximization of variance. For example, a Gaussian distribution can be obtained from such principles if limits of change of variable are taken to be infinite, and the mean and variance are assumed to be known. We consider the distribution given by Equation A7 as an acceptable one because it doesn't contradict the expectations formulated in our second argument. By changing the parameters of this distribution, one can probe the influence of age interval uncertainties on final results. For example, if $\alpha = 0$, the distribution becomes uniform. We took the parameter X_{max} to be equal to 15 hr for both $\hat{x}_{L_1}$ and $\hat{x}_{add}$. The parameter α was calculated to fit the mean values of $\hat{x}_{L_1}$ and $\hat{x}_{add}$, which were both 3 hr.

To obtain estimates of the sampling variance of the demographic parameters resulting from measurement error, we obtained 1000 Monte Carlo estimates of each of them for each individual. The average of the within-individual variation was then taken to be an estimate of the variance resulting solely from constraints on our measurement abilities. By subtracting this variance from the within-line variance estimates obtained by analysis of variance of the individual measures, we obtained estimates of the error variance caused by environmental causes. This procedure only influences our estimates of mutational heritability because none of the other parameter estimates depends on the within-line variance. The simulations showed that no more than 5–20% of the within-line variance was a consequence of measurement error, so even in the absence of such a correction, the mutational heritabilities would only be underestimated by a small fraction.