Spontaneous Mutational Variation for Body Size in *Caenorhabditis elegans*

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ABSTRACT

We measured the impact of new mutations on genetic variation for body size in two independent sets of *C. elegans* spontaneous mutation-accumulation (MA) lines, derived from the N2 strain, that had been maintained by selfing for 60 or 152 generations. The two sets of lines gave broadly consistent results. The change of among-line genetic variation between cryopreserved controls and the MA lines implied that broad sense heritability increased by 0.4% per generation. Overall, MA reduced mean body size by ~0.1% per generation. The genome-wide rate for mutations with detectable effects on size was estimated to be ~0.0025 per haploid genome per generation, and their mean effects were ~20%. The proportion of mutations that increase body size was estimated by maximum likelihood to be no more than 20%, suggesting that the amount of mutational variation available for selection for increased size could be quite small. This hypothesis was supported by an artificial selection experiment on adult body size, started from a single highly inbred N2 individual. We observed a strongly asymmetrical response to selection of a magnitude consistent with the input of mutational variance observed in the MA experiment.

THE contribution of spontaneous mutations to the variability of a quantitative trait can be quantified as the mutational variance, V_m, the new genetic variance arising in one generation (Clayton and Robertson 1955). To compare the mutability of different traits or the same traits across species, the mutational variance is often scaled by dividing by the environmental variance of the trait and expressed as the mutational heritability, h_m^2. Mutational heritabilities for many traits of multicellular eukaryotes are frequently in the range 10^{-3}–10^{-2} (Houle *et al.* 1996), and there is evidence for a positive correlation between mutational heritability and generation time (Lynch *et al.* 1999). Knowledge of mutational variation or heritability is useful on its own for predicting rates of response to artificial selection in the long term (Hill 1982a,b) or the genetic variance and divergence of populations under the neutral model (Lynch and Hill 1986); however, the evaluation of models involving most forms of selection requires information on the rates and distributions of effects of mutations (Burger 2000).

Two ways to study the properties of spontaneous mutational variation for quantitative traits are by long-term selection starting from an inbred ancestral line or by mutation accumulation (MA) in closely inbred lines under relaxed selection. Long-term artificial selection experiments have the advantage of potentially rapidly fixing mutational differences between lines and have told us much about the potential of new mutations to lead to selection response (*e.g.*, Lopez and Lopez-Fanjul 1993a; Mackay *et al.* 1994) and about the properties of mutations that contribute (Lopez and Lopez-Fanjul 1993b; Fry *et al.* 1995; Mackay 1996; Mackay and Fry 1996). However, large-effect mutations make disproportionate contributions to selection response, and inferences are restricted to the traits under selection. MA experiments, in which mutations are allowed to accumulate in lines of small effective size under relaxed selection, can provide a clearer picture of the properties of new mutations that is less biased by selection. This is currently the only way that is available to estimate the rates and average effects of new mutations for quantitative traits (Crow and Simmons 1983) or parameters of the distribution of mutational effects (Keightley 1994; Garcia-Dorado 1997; Shaw *et al.* 2002).

In the nematode *Caenorhabditis elegans*, a species that normally reproduces by self-fertilization, there have been two spontaneous MA experiments carried out (Keightley and Caballero 1997; Vassilieva and Lynch 1999). Studies of fitness-related traits of the MA lines and frozen control populations indicate that spontaneous mutation accumulation has an overwhelmingly negative effect on fitness components and that the distributions of mutational effects appear to be somewhat platykurtic. Mutant alleles that are detectable on the

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basis of subline divergence for most life-history traits occur at a rate of the order of 0.01 per haploid genome per generation and have average homozygous effects of ~20% (Keightley and Bataillon 2000; Vassilieva *et al.* 2000), but these rate (average effect) estimates are likely to be substantially biased downward (upward, in absolute value), due to the presence of deleterious mutations that have negligible effects in laboratory conditions (Davies *et al.* 1999) and the inherent limitations of biometrical analysis methods (Keightley 1998a; Lynch and Walsh 1998).

Here, we report on experiments in which we have assayed the above spontaneous MA lines for body size. We estimate the rate of accumulation of mutations for body size and properties of the distribution of their effects using methods that rely on the moments of the genotypic distribution or by maximum likelihood (ML). We also report on the results of an artificial selection experiment on body size in a selfing population of the same strain of *C. elegans* as was used to initiate the MA experiments. We compare the two experiments by modeling the selection experiment, while assuming mutational parameters that we estimated from the MA experiment.

MATERIALS AND METHODS

Strains, culture conditions, and freezing: Three independent sublines of the Bristol N2 strain of *C. elegans* were used in the experiments, all originally obtained from the *Caenorhabditis* Genetics Center (St. Paul, MN): one for each set of MA lines (see below) and one for the artificial selection experiment. All strains were maintained at 20° in 9-cm NGM agar plates seeded with a lawn of *Escherichia coli* strain OP50 (Sulston and Hodgkin 1988). Strains were frozen at −80° in a glycerol solution using standard procedures (Sulston and Hodgkin 1988).

Mutation accumulation: The procedures used to generate the MA lines have been discussed in detail in the original reports (Keightley and Caballero 1997; Vassilieva and Lynch 1999). A series of 50 MA lines in the Keightley and Caballero (KC) experiment and a series of 100 strains in the Vassilieva and Lynch (VL) experiment were founded (independently in each experiment) by the descendants of a single inbred individual derived from the N2 strain of *C. elegans*. Each MA line was maintained for several generations by transfer of a single individual larva picked at random.

For this study we assayed 48 lines from the KC set that had accumulated mutations for 60 generations and 69 lines from the VL set that had accumulated mutations for an average of 152 generations (SD = 3.7). These strains (denoted KC_{MA} and VL_{MA}, respectively) and two replicates (1 and 2) of the corresponding KC and VL ancestral strains (denoted KC_C and VL_C, respectively) were obtained from the different laboratories, kept at high density for 3–5 generations, and then frozen in A. M. Leroi's laboratory (Sulston and Hodgkin 1988).

The lines were revived from freezing and allowed to expand to high density over three to four generations. All lines were assayed simultaneously and concurrently with four sets of 10 control lines derived from the KC_{C1}, KC_{C2}, VL_{C1}, and VL_{C2} replicates of the ancestral strains (one VL_{C1} subline was accidentally lost). Before the assay, each of these 156 lines was split into three replicates that were maintained for two generations by single-worm transfer. Finally, with the strains approximately synchronized, one 96-hr-old individual per replicate was allowed to lay eggs on a fresh plate for ~1 hr. Eighteen to 24 hr later 20 larvae per replicate were transferred to a fresh plate and, 72 hr after egg laying (we did not detect any differences among lines in hatching time), 10–15 of these worms were collected into centrifuge vials (1 per replicate) containing a fixative (4% glutaraldehyde in PBS buffer). From each replicate, 5 worms were randomly picked out of the fixative and mounted on an agar pad with a drop of PBS buffer and photographed at ×50 magnification.

We have also used previously collected data on hermaphrodite self-fertility and lifespan in the KC (generation 60, Keightley and Caballero 1997) and VL lines (generation 163, Vassilieva *et al.* 2000) to investigate whether mutations that affected body size had pleiotropic effects on fitness.

Artificial selection on new mutations: A single inbred individual of the Bristol N2 strain of *C. elegans* was used to found a new line, which was maintained for three generations by single-individual transfer. The population was then allowed to expand, and each of three lines (designated control, high, and low) was established by immersing 20 72-hr-old individuals in a sodium hypochlorite solution (Sulston and Hodgkin 1988) and allowing the resulting eggs to hatch (a procedure referred to as bleaching). Descendants from this ancestral line were frozen. Every generation, 96 individuals of the high line were randomly transferred to a fresh unseeded agar plate, allowed to move for a few seconds, photographed at ×25 magnification, and finally, collected into 12-well plates containing M9 buffer (Sulston and Hodgkin 1988). The worms were measured (see below), and then the 20 individuals showing the largest volume were selected and bleached on a fresh plate, giving rise to the following generation. A similar procedure was applied to the low line, except that the 20 individuals with the smallest volume were selected. In the control line, 20 individuals were collected at random. The procedure was repeated each generation. Every 6 generations, descendants of all three lines were frozen. Selection was continued for 48 generations.

At the end, the ancestral line and all lines from generations 12, 24, 36, and 48 were revived from freezing and split into three replicates derived from three to five individuals. The replicates were allowed to expand to high density over 2–3 generations. Before the assay, each replicate was propagated for 2 generations by bleaching 20 hermaphrodites and allowing the eggs to develop normally. Finally, 10 96-hr-old hermaphrodites per replicate were allowed to lay eggs in a fresh plate for ~1 hr. Twenty to 28 hr later 20 larvae per replicate were transferred to a fresh plate and, 72 hr after egg laying (no significant differences among lines in hatching time were detected), 10 hermaphrodites were randomly transferred to a fresh agar plate without food and photographed at ×25 magnification. The remaining hermaphrodites were photographed at 120 hr in the same way.

In parallel to the previous experiment, adult hermaphrodites from the ancestral line and all lines from generation 48 were allowed to lay eggs onto fresh plates for 2 hr. For each line, 25 hatchlings were transferred to individual plates 14–16 hr after egg laying. Each worm was transferred daily to a freshly seeded plate and the number of viable progeny was counted 24–48 hr later. The life span of each worm was recorded.

Worm measurements: Individual worms were photographed using a Leica dissecting microscope with a JVC KY-F50 video camera connected to a Power Macintosh computer, running the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet

at http://rsb.info.nih.gov/nih-image). In the MA line assay each individual was measured by tracing its outline using the "Poly" Object type in Object-Image (Vischer *et al.* 1994; available on the Internet at http://simon.bio.uva.nl/object-image.html). In the selection experiment and its assay, each picture was subjected to the "Convolve" command with the Hat (13 × 13) kernel distributed with NIH Image. The outline of each worm was closed manually and then was selected automatically (using the "AutoThreshold" and "AutoOutline" commands) and measured. The cross-sectional area (A) and perimeter (P) of the worm were thus obtained, and these measurements were used to estimate body volume (S), under the assumption that the worm is cylindrical:

$$S = \frac{\pi(P + \sqrt{P^2 - 16A})\ (P - \sqrt{P^2 - 16A})}{256}.$$

Body volume was expressed as $S \times 10^3$ mm^3 throughout, for computational convenience. Note that the measurements in the assays of the MA and selection lines are not directly comparable, because the worms in the former were fixed in glutaraldehyde, which causes the worms to shrink by ~30% in volume (CV = 4.7%, $N = 18$), independently of initial size.

Analysis of the MA experiments: In each of the four sets of lines, we fitted a nested linear model to the individual measurements, with line and replicate within line as random effects, and estimated the components of variance between lines (V_L) and within replicates (the environmental variance, V_e) by restricted maximum likelihood (REML). The residuals of individual measurements were normally distributed in the VL_C lines (Shapiro-Wilk test, $P > 0.1$), but not the KC_C, KC_{MA}, and VL_{MA} lines ($P < 0.0005$). The increase in genetic variance per generation due to mutation was estimated as $V_m = V_L/(2t)$, where t is the number of generations of mutation accumulation. The change in the mean per generation was calculated as $\Delta M = [M_{MA} - M_C]/t$, where M_C and M_{MA} are the means of the control and MA lines, respectively. The haploid genomic mutation rate per generation (U) and the average mutational effect (a) in the homozygous state were estimated by the Bateman-Mukai (BM) method (Bateman 1959; Mukai 1964), in which mutation effects are assumed to be equal:

$$U_{BM} = (\Delta M)^2/(2V_m)$$

$$a_{BM} = 2V_m/\Delta M.$$

Approximate SEs for the linear model parameters (M, V_e, and V_L) were obtained using a normal approximation to the REML estimators; approximate SEs for other statistics were calculated as the median absolute deviations (a robust estimator of the SD) of the statistic calculated on 1000 bootstrapped data sets at the level of line. The above analyses were done using the statistical software R (Ihaka and Gentleman 1996).

Estimates of the genomic mutation rate and parameters of the distribution of mutational effects were also obtained by maximum likelihood as described in detail previously (Keightley 1994, 1998a). Briefly, the phenotypic value of each replicate mean was assumed to be a normally distributed random environmental effect with mean M and variance V_e minus a genetic effect g. The calculations were carried out on replicate means rather than individual values to reduce the problem of nonnormality of the residuals of individual values (see above). The residuals for replicate means deviated significantly from normality only in the case of the KC_{MA} lines ($P < 0.005$). The environmental variance was found to differ significantly between VL_C and VL_{MA} lines, so separate V_e were fitted for control and MA lines. The value of g was the sum of n_g random deviates from the distribution of mutational effects (the parameters of which are estimated), where n_g is a random integer from a Poisson distribution with parameter Ut. The distribution of mutational effects was assumed to be gamma, which allows a wide variety of shapes. The distribution has two parameters, α specifying scale and β specifying shape. The mean of the distribution is $E(a) = \beta/\alpha$. A very large value for β implies a distribution with a variance close to zero and so is asymptotically equivalent to the equal effects model assumed under the BM method, while small values of β imply leptokurtic distributions. We employ the strategy of obtaining estimates of U and $E(a)$ for a variety of distributions and comparing the fit of the different distributions to the data via likelihood.

The basic analysis described above assumes that mutations have unconditionally negative effects on body size and that g is therefore positive. We also investigated the fit to the data of models in which the distribution of mutational effects follows a reflected gamma distribution with a proportion R of mutations having an increasing effect on the trait and a proportion $1 - R$ decreasing. In the full model, the parameters to be estimated were M, the two V_e, U, β, $E(a)$, and R.

Approximate SEs for the ML estimates were calculated from the curvature of likelihood about the maxima (Weir 1996).

Predicting the selection response: In a selection experiment involving an outcrossing species starting from an inbred ancestral strain, it is possible to obtain estimates of the mutational variability, V_m, by regression of the observed cumulative response to selection against the expected response according to genetic models that make different assumptions about the nature of the underlying genetic variation (Hill 1982b). A general solution for the asymptotic selection response under an additive model applies both to the infinitesimal model and to a major genes model, whereas the initial rate of response depends on the genetic details (Hill 1982b). The selection response from new mutations under systems of mating involving partial inbreeding has been theoretically investigated by Caballero and Hill (1992) and Caballero and Santiago (1995). In the case of a selfer such as *C. elegans*, the simple predictions of response under artificial selection from new mutations is impossible, principally because selective sweeps of advantageous mutations greatly reduce the effective population size. Since the effect of selective sweeps on background genetic variation depends on the effects and genome-wide rate for new mutations, general solutions for the initial or asymptotic selection responses have not been obtained.

Therefore we investigated whether the results from the MA and the artificial selection experiments accorded with one another by Monte Carlo simulation of the selection experiment. We simulated truncation selection with a Poisson distribution of family size assuming unlinked new mutations occurring throughout the genome. Mutations occurred in the progeny and had an immediate effect on phenotypic value (hence affecting the probability of selection in the generation in which they occurred). The order of events in the simulation was therefore mutation, selection, recombination. We also examined the effect of variation of the degree of dominance of new mutations.

RESULTS

Mutation accumulation—basic statistics and BM analysis: In both the KC and VL lines (see materials and methods), mean body volume decreased with the accumulation of spontaneous mutations (Figure 1). Mean body size declined by $\Delta M/M_C = -0.02\%$ per generation in the KC lines (one-tailed permutation test, $P > 0.3$) and by -0.06% per generation in the VL lines ($P < 0.05$; Table 1). Note that, on average, the KC control lines were 20% larger than the VL control lines ($P <$

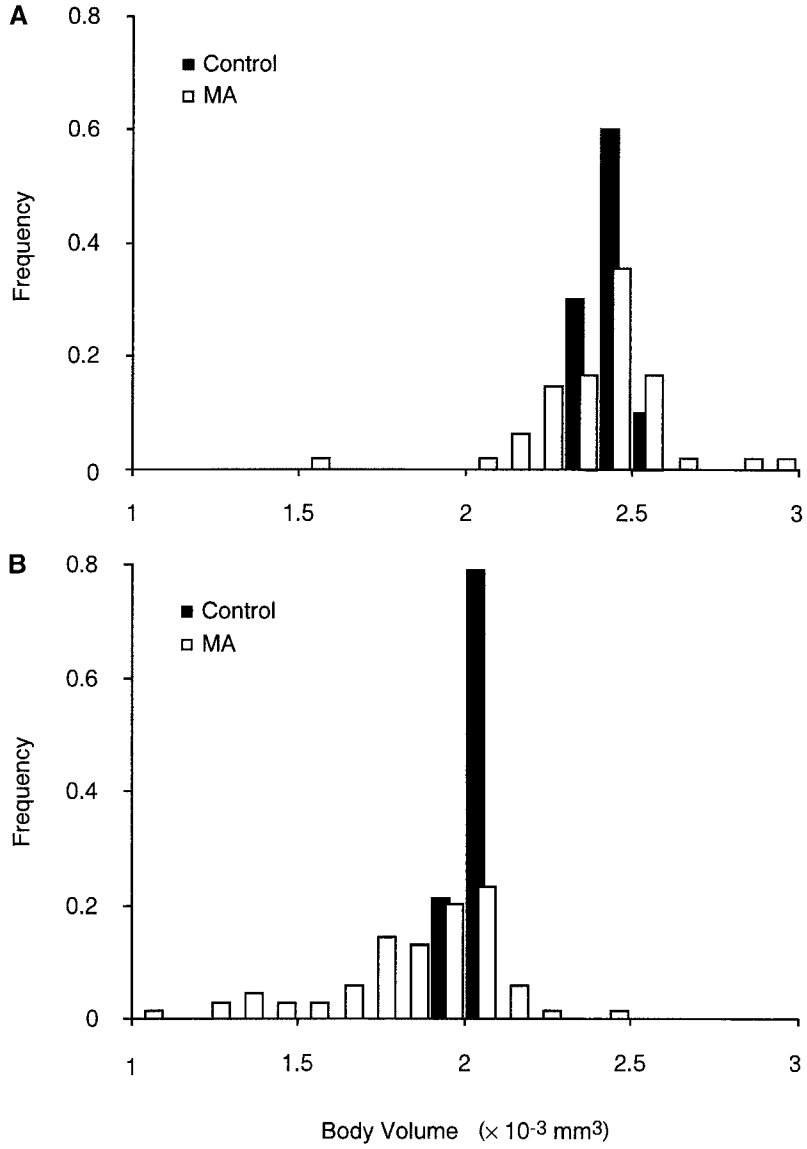

FIGURE 1.—Distributions of mean body volumes in control (solid bars) and mutation-accumulation lines (open bars) of *C. elegans*. Both the KC (A) and VL (B) sets were assayed simultaneously (total number of individuals measured, $N = 2178$).

0.0001), even though they were both derived from Bristol N2 (Figure 1). Considerable variation among other N2 sublines has also been detected for longevity (GEMS and RIDDLE 2000).

In both the KC and VL lines, the among-line variance (V_L) of body volume increased with the accumulation of spontaneous mutations (Figure 1; Table 1). The among-line variance component was nonsignificant in both sets of control lines (ANOVA, $P > 0.1$) but significant in both sets of MA lines ($P < 0.0001$). The genetic variance (V_m) increased by $\sim 0.4 \times 10^{-3}$ per generation due to mutation in the two sets of lines (Table 1). The mutational heritability was $h^2_m = V_m/V_e \approx 0.4\%$ and the mutational coefficient of variation was $CV_m = \sqrt{V_m}/M_C \approx 1\%$. The environmental variance (V_e) of body volume increased with the accumulation of spontaneous mutations in the VL experiment (F-test, $P < 0.05$), but did not change significantly in the KC experiment ($P > 0.05$).

In the VL lines, using the BM analysis, the haploid genomic mutation rate per gamete per generation was estimated to be $U_{BM} = 0.0018$, while the mean homozygous mutational effect on body size was estimated to be $a_{BM}/M_C = -32\%$ decrease in body size (Table 1). The estimates of mutational parameters for the KC lines ($U_{BM} = 0.0003$ and $a_{BM}/M_C = -76\%$) are more imprecise because ΔM is nonsignificant (Table 1). Presumably, this is a consequence of 2.5 times fewer generations of MA and the smaller number of lines analyzed in the KC compared to the VL experiment.

Body volume was positively correlated with fertility among MA lines in both experiments (Table 1; KC, permutation test, $P > 0.1$; VL, $P < 0.05$; combined significance by Fisher's method, $P < 0.05$). The V_m for life span in the KC experiment was nonsignificant (KEIGHTLEY and CABALLERO 1997). Body size was not significantly correlated with life span among the VL_{MA} lines ($r = -0.09$, SE = 0.13, $P > 0.4$).

TABLE 1

Basic statistics from the mutation-accumulation experiments

Parameter	Experiment	
	KC	VL
M (controls)	2.376 (0.030)	1.956 (0.030)
M (MA)	2.346 (0.039)	1.788 (0.045)
V_e (controls)	0.105 (0.011)	0.100 (0.010)
V_e (MA)	0.087 (0.005)	0.122 (0.006)
V_L (controls)	0.007 (0.008)	<0.001 (>1)
V_L (MA)	0.055 (0.016)	0.105 (0.025)
$\Delta M \times 10^3$	−0.507 (0.789)	−1.106 (0.351)
$V_m \times 10^3$	0.460 (0.131)	0.345 (0.082)
CV_m (%)	0.903 (0.247)	0.949 (0.117)
h^2_m (%)	0.438 (0.245)	0.345 (0.091)
U_{BM}	0.00029 (0.00307)	0.00177 (0.00403)
a_{BM}/M_C	−0.763 (0.272)	−0.319 (0.043)
r	0.229 (0.222)	0.318 (0.130)

Values are estimates (SE) of the following statistics: M, mean body volume; V_e, environmental variance; V_L, among-line variance; ΔM, rate of decline in mean body volume per generation; V_m, mutational variance; CV_m, coefficient of mutational variation; h^2_m, mutational heritability; U_{BM} and a_{BM}, haploid genomic mutation rate per generation and average homozygous mutational effect, under an equal effects model; r, phenotypic correlation between body size and fertility (MA line means).

TABLE 2

Estimates of mutational parameters from ML analysis assuming a one-sided gamma distribution

Model (β)	Estimate (SE)		Fit
	U	$E(\|a\|)/M_C$	
KC			
→ ∞	0.0022 (0.0014)	0.20 (0.036)	−0.5
2	0.0031 (0.0023)	0.15 (0.068)	−0.0
1	0.0041 (0.0031)	0.11 (0.058)	−0.0
0.25	0.010 (0.0081)	0.048 (0.029)	−0.1
0.0625	0.034 (0.0290)	0.014 (0.010)	−0.2
VL			
→ ∞	0.0027 (0.0008)	0.24 (0.035)	−0.4
2	0.0033 (0.0011)	0.16 (0.050)	−0.3
1	0.0045 (0.0016)	0.15 (0.045)	−0.8
0.25	0.011 (0.0043)	0.060 (0.022)	−1.6
0.0625	0.039 (0.0160)	0.018 (0.008)	−2.0

Fit is the difference in log-likelihood of the model with the value of β shown from the best-fitting model, the parameters of which are given in the text.

Mutation accumulation—ML analysis: Under ML, the model with equal mutational effects (β → ∞) corresponds to the model assumed under the BM analysis. In the case of the VL experiment, the ML and BM estimates of U and a_{BM}/M_C are reasonably close to one another, but the parameter estimates agree rather poorly in the case of the KC experiment (Tables 1 and 2). However, the ML analysis under the equal effects model gives estimates that are remarkably similar in both experiments with considerably smaller sampling variances than the BM analysis. This is due to a more efficient use of the available information on the distribution of the data under ML (Keightley 1998a). Both experiments point to low rates of mutation affecting body size (∼0.0025 per haploid genome per generation), comprising mutations with large effects (∼20%).

The ML analysis also allowed the fit of a variety of gamma distributions with different shape parameters (β) to be compared (Table 1). As has been the case with several other MA experiments in which ML or minimum distance methods (Garcia-Dorado 1997) have been employed, the fit to the data (measured in the case of ML by log-likelihood) does not change a great deal as a function of β. Thus, the data from the experiments are compatible both with the equal effects model and models involving highly leptokurtic distributions of mutational effects in which the mean effect is very small, but most of the variance is contributed by mutations of large effect. These latter models also imply much higher mutation rates than the equal effects model (Table 1). However, in the KC experiment the best-fitting one-sided gamma distribution has a β parameter of 1.2 and a mean homozygous effect of 12%, while the corresponding estimates from the VL experiment are β = 5 and mean effect $E(a)$ = 25%. The best-fitting models therefore imply platykurtic distributions of mutational effects, although it is not possible to discriminate between these models and models involving bimodal distributions of mutation effects (Davies *et al.* 1999).

The fit of reflected gamma distributions was investigated with the shape parameter β fixed at 2, a value that gives a good fit to both data sets under the one-sided model (see Table 2). Investigation of a range of models with different values of β was not feasible, due to the high computational demands of the likelihood evaluation. In the case of the KC data set, the best-fitting reflected gamma has a proportion of positive effects parameter (R) of 0.30. However, there is very little information to distinguish models with different R values, since the fit of models with R = 0 and R = 0.5 is not significantly poorer (Table 3). In the case of VL, an unreflected distribution in which all mutations have decreasing effects fits the data best. The highest value compatible with the data, based on a likelihood-ratio test, is $R \approx 0.2$ (Table 3). These results suggest that the distribution of mutational effects on body size is skewed downward.

Response to artificial selection: The measurements taken during the selection experiment suggested that both selected lines diverged gradually from the control line (Figure 2A). This was confirmed by the final assay

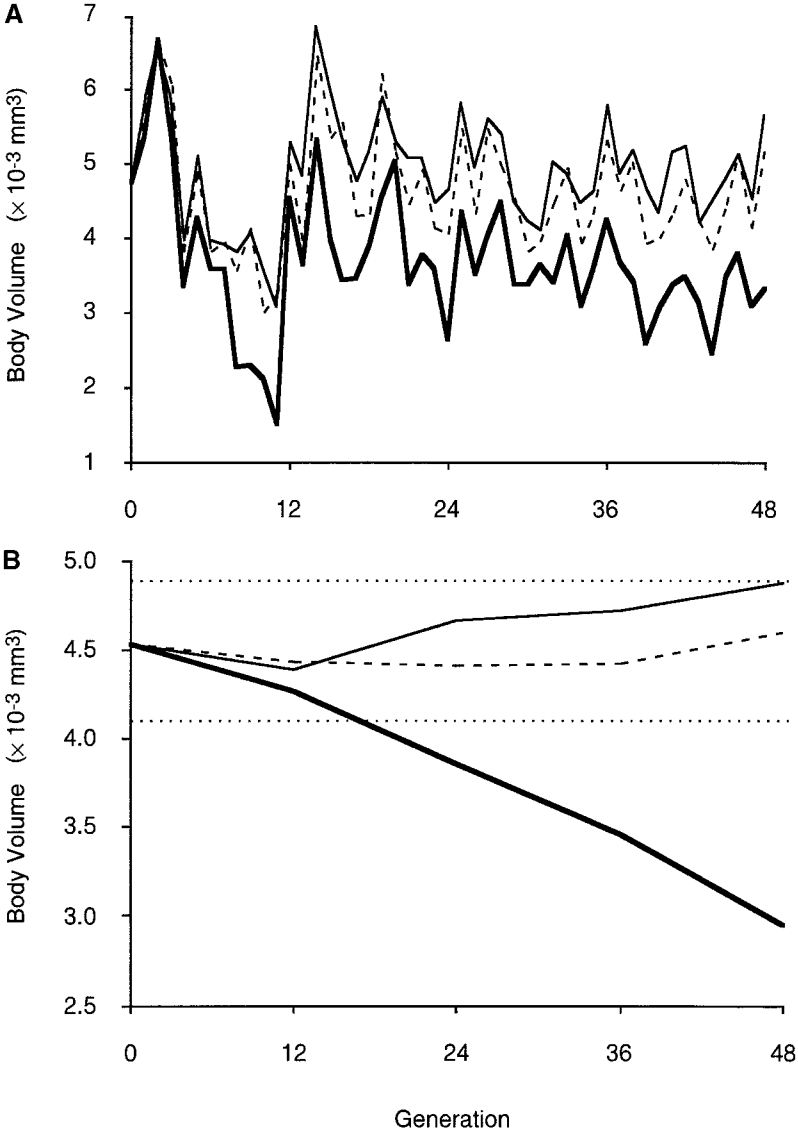

FIGURE 2.—Response to selection for body volume on new mutations in *C. elegans*. (A) Mean body volume in each line during the selection experiment (total number of individuals measured, $N = 13{,}647$). (B) Mean body volume of each line at generations 12, 24, 36, and 48, assayed simultaneously (total number of individuals measured, $N = 760$). The responses of the high, low, and control lines are indicated by solid, thick, and dashed lines, respectively. The dotted lines denote the mean $\pm \sigma_P$ of the ancestral and control lines during selection.

(Figure 2B): The control line did not change significantly in body volume during the selection experiment (linear orthogonal contrast in one-way ANOVA on replicate means, $P > 0.5$), whereas both the high ($P = 0.001$) and low ($P < 0.0001$) lines diverged significantly. At generation 48, the high line had increased by 8% and the low line had decreased by 35%.

In *C. elegans*, adult hermaphrodites continue to grow in volume and attain maximum body size at 100–140 hr. The selected lines also diverged in body size at 120 hr (data not shown). Fitting the allometric equation $S_{120} = a \cdot S_{72}^b$ (where S_{72} and S_{120} are the mean body sizes at 72 and 120 hr, respectively) to the line means at each generation ($N = 13$) showed that b was not significantly different from 1 (nonlinear least-squares, $b = 1.0$, SE = 0.11) and, therefore, that selection changed body size at the two times isometrically. Fitting an allometric equation with $b = 1$, we obtained $a = 1.40$ (SE = 0.016), which means that worms grew, on average, by 40% in volume between 72 and 120 hr.

The selected lines did not diverge significantly in fertility (Kruskal-Wallis test comparing all lines, $P > 0.2$; Table 4) or life span (log-rank test comparing all lines, on censored observations, $P > 0.1$; Table 4). However, even though the low line did not diverge in the total number of offspring produced, it evolved a more protracted egg-laying schedule: Whereas hermaphrodites in the ancestral, control, and high lines lay only ~5% of their fertilized eggs after 120 hr from hatching, those from the low line laid 14% of their fertilized eggs in that period (Kruskal-Wallis test comparing all lines, $P < 0.001$; Table 4).

Comparison of the mutation-accumulation and selection experiments: We tested the agreement between the genetic changes observed in the MA and selection experiments by simulation. We investigated two models, the first based on parameter estimates from the MA experiments (Table 3), and the second with a high mutation rate comprising mutations with small effects that generated the same mutational variance. We as-

TABLE 3

Estimates of mutational parameters from ML analysis assuming a reflected gamma distribution

Experiment	R	U	$E(\|a\|)/M_C$	log L ($R = 0$)	log L ($R = 0.5$)
KC	0.3	0.0058	0.13	−1.1	−0.9
VL	0	0.0033	0.20	0	−8.6

sumed a reflected gamma distribution with shape parameter 2 and the average of the R estimates from the MA experiments (*i.e.*, 0.15, Table 3) and compared the control–low divergence with the simulations. Since the traits measured in the MA and selection experiments were somewhat different, we assumed that mutational effects were of the same magnitude when expressed on the scale of phenotypic SD (σ_P) units, by scaling with the average of the $\sqrt{V_e}$ of the control lines in the MA experiments (1.10, Table 1) and low selection line (0.430). The small additive mutational effects model gives the best fit to the observations (Figure 3, Table 5), but responses under the large gene effects models with either recessive or additive mutations are highly variable, and the observed response is compatible with both these models (Table 5). In the case of the small recessive gene effects model, the fit to the observed response only approached significance: Only 5% of simulated responses exceeded the observed response of 1.64 at generation 48.

DISCUSSION

Mutational variation for body size: Assays of the two independent sets of *C. elegans* MA lines gave estimates for mutational parameters that are in good agreement with one another. The mutational heritability for body size was $\sim h_m^2 = 0.4\%$ per generation, a figure somewhat higher than previously reported for other life history traits in *C. elegans* (mostly in the range 0.1–0.3%; Keightley and Caballero 1997; Vassilieva *et al.* 2000), but similar to estimates for body size in other species [*D. melanogaster*, 0.2% for wing dimensions (Santiago *et al.* 1992), 0.02% for thorax length (Wayne and Mackay 1998); *Daphnia pulex*, 0.3% (Lynch 1985); mouse, 0.5% (Keightley 1998b)]. The rate of change of mean body size due to spontaneous mutation accumulation was $\sim -0.05\%$, which is similar to the rate of mutational decay for several life history traits measured under standard laboratory conditions (Keightley and Caballero 1997; Vassilieva and Lynch 1999).

TABLE 4

Correlated selection responses at generation 48

Line (N)	Life span[a]	Fertility	Proportion of viable eggs laid after 120 hr (%)
Ancestral (23)	13.2 (0.83)	265 (16)	5.2 (1.4)
Control (22)	11.9 (0.95)	279 (14)	5.9 (0.9)
Low (25)	13.7 (0.99)	252 (11)	13.8 (1.5)***
High (25)	14.7 (1.04)	263 (12)	4.6 (1.0)

Each selected line was compared to the ancestral line by a Wilcoxon signed-rank test. Significance levels: ***$P < 0.001$; others, $P > 0.05$. Unless otherwise stated, values are means (SE).

[a] Kaplan-Meier product-moment estimates (SD).

Distribution of mutational effects: The estimates for genome-wide rates of mutation are also consistent with the surprisingly low estimates previously obtained for several life history traits (Keightley and Caballero 1997; Keightley and Bataillon 2000; Vassilieva *et al.* 2000). We estimated rates and effects of mutations by the BM method of moments and by ML. BM mutation rate estimates assume unidirectional mutations with equal effects and tend to be biased downward, since mutational effects will vary (Lynch and Walsh 1998); however, the extent of this bias is unclear. A different *C. elegans* MA experiment involving ethyl methanesulfonate (EMS) mutagenesis, for which the rate of point mutation in the genome could be calibrated, suggested that the genome-wide mutation rate estimated from the genotypic distribution could be >10 times too low (Davies *et al.* 1999; Keightley *et al.* 2000). This would also imply substantial variation among mutation effects. However, the calibration depends on assuming a specific rate for EMS-induced point mutations, and these have been obtained for a limited number of sites in the *C. elegans* genome (Anderson 1995). Better information on the number of deleterious mutations actually arising in MA lines may ultimately be obtained by combining information on the overall level of selective constraint in the genome (*e.g.*, Shabalina and Kondrashov 1999) with direct molecular estimates of the rate of accumulation of molecular variants (Denver *et al.* 2000).

ML analysis allows the fit of alternative distributions of mutational effects to be compared, as well as simultaneous estimation of U and the average homozygous mutational effect. These are the parameters that we would like to accurately estimate from MA experiments. However, in practice, the information content is low, so likelihood profiles for parameters of the distribution of mutational effects are rather flat. The ML estimates suggest that the distribution of mutational effects could be platykurtic, a result we also observed for several life history traits (Keightley and Bataillon 2000; Vassilieva *et al.* 2000). Since the true number of mutations that have phenotypic effects at some level may have been

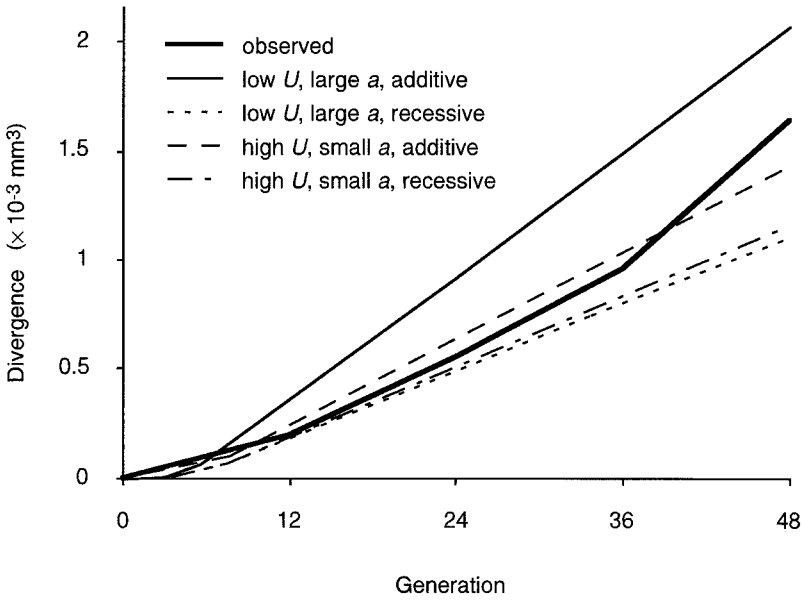

FIGURE 3.—Comparison of the response to selection for small body size with results from Monte Carlo simulations that assumed similar parameter values as estimated from ML analysis of MA experiments (Table 4) or assuming many mutations with small effects. A reflected gamma distribution of mutational effects with the proportion of positive mutations $R = 0.15$, the average of the estimates from the MA experiments (Table 4), was assumed. The mutation rate per haploid genome was assumed to be $U = 0.00485$ (the average of the estimates for the reflected gamma distribution, Table 4) or 0.1, and the homozygous mutational effect was assumed to be $a = 1.10$ or $0.241\ \sigma_P$ units (Table 4), implying that effects on body size in the selection experiment are $a = 0.473 \times 10^{-3}$ or 0.104×10^{-3} mm^3. Mutations were either recessive or additive. The lines are averages of 1000 simulation replicates.

drastically underestimated (see above), the inference of platykurtic distributions of mutational effects with relatively high means (of the order of 20%) could imply that the outcome of the likelihood analysis (and BM calculations) is heavily influenced by the presence of bimodality or multimodality in the distribution of mutational effects (SHAW *et al.* 2002). This relates to a fundamental problem with any biometrical method for estimating gene numbers and effects (*e.g.*, the Castle-Wright index), which fails to detect mutations with small effects.

Response to artificial selection: The two MA experiments and the response to artificial selection on body size together provide evidence that spontaneous mutations have a greater overall effect decreasing than increasing body size. Only 10–20% of the overall mutational effect is in the upward direction. In *Drosophila melanogaster* the majority of large-effect spontaneous mutations detected in MA lines decrease wing length (SANTIAGO *et al.* 1992), a trait closely correlated with body size. Similarly, in a study of the effect of EMS mutagenesis on various quantitative traits in *D. melanogaster*, KEIGHTLEY and OHNISHI (1998) observed a significant reduction in body size of treated lines relative to controls and inferred that the maximum overall proportion of increasing-effect mutations was 18%. More recently YANG *et al.* (2001), also studying the effects of EMS on quantitative traits in *D. melanogaster*, reported negative effects on body size. Taking all of the above evidence together, the input of new mutational variation that has the potential to fuel upward artificial selection (the direction that is of most interest to breeders) could be as much as an order of magnitude lower than the variance that can be used for downward selection. In addition, mutations with large effects on quantitative traits frequently have deleterious effects on components of fitness (LYMAN *et al.* 1996), so the amount of mutational variation that can fuel selection response for increased size may be reduced still further, perhaps to far below the figure of $0.001 \times V_e$ that is often assumed (HILL 1982a,b; FALCONER and MACKAY 1996). One experiment that seems to contradict this view is a long-term artificial selection experiment on body weight in mice (KEIGHTLEY 1998b), in which the response to upward selection was about twice as great as the downward response. Crossing experiments between the mouse lines suggested that the upward response was due to one or two large-effect mutations. The stochastic nature of response from new mutations might reconcile this experiment with the others.

The asymmetry of response to selection from new mutations described here contrasts with the results of artificial selection experiments in outbred populations. Responses to selection are generally asymmetrical for life history traits (FRANKHAM 1990), but tend to be fairly symmetrical for body size, in a variety of organisms (*e.g.*, DINGLE *et al.* 1988; PARTRIDGE and FOWLER 1993). Several artificial selection experiments for components of body size have been conducted in outbred populations of Drosophila (Table 6). There is a significant tendency for the response downward to be faster than upward (paired Wilcoxon signed rank test, $P < 0.05$), by ~25% on average. But the asymmetric selection responses seen

TABLE 5

Simulated selection response at generation 48

U	$E(a)$	Gene action	Simulated response[a]
0.00485	0.473	Additive	2.11 (0.53, 3.77)
0.00485	0.473	Recessive	1.13 (−0.02, 2.40)
0.1	0.104	Additive	1.46 (0.93, 2.00)
0.1	0.104	Recessive	1.19 (0.73, 1.74)

[a] Means (2.5, 97.5 percentiles) of 1000 simulation replicates.

TABLE 6

Survey of realized heritability estimates from artificial selection on components of body size in outbred populations of Drosophila

Trait (reference)	Replicate lines	Realized heritability		
		High line	Low line	$h^2_H - h^2_L$
Body size index (1)	2	0.14	0.20	−0.06
Body weight (2)	4	0.18	0.23	−0.05
Body weight (2)	4	0.17	0.14	0.03
Thorax length (3)	1	0.39	0.27	0.12
Thorax length (3)	1	0.32	0.26	0.06
Thorax length (3)	1	0.25	0.34	−0.09
Thorax length (4)	1	0.28	0.44	−0.16
Thorax length (5)	2	0.25	0.25	0.00
Thorax length (6)	2	0.14	0.12	0.02
Thorax length (6)	2	0.11	0.19	−0.08
Thorax length (6)	2	0.13	0.14	−0.01
Wing area (7)	1	0.58	0.59	−0.02
Wing area (8)	3	0.42	0.47	−0.05
Wing length (9)	1	0.03	0.12	−0.09
Wing length (9)	1	0.18	0.22	−0.04
Wing length (10)	2	0.36	0.41	−0.04
Wing length (10)[a]	2	0.27	0.33	−0.05
Wing length (11)	2	0.18	0.54	−0.36
Wing length (11)	2	0.29	0.40	−0.11
Wing length (11)	2	0.22	0.17	0.05
Wing length (11)	2	0.26	0.25	0.01
Wing length (11)	2	0.12	0.48	−0.35
Mean (SE)		0.24 (0.026)	0.30 (0.030)	−0.06 (0.024)

Estimates are based on independent selection experiments in *D. melanogaster* and *D. simulans*.

[a] References: (1) Baptist and Robertson (1976); (2) Hillesheim and Stearns (1991); (3) Robertson (1955); (4) Masry and Robertson (1978); (5) Scheiner and Lyman (1991); (6) Reeve and Fairbairn (1996); (7) Partridge *et al.* (1999); B. Zwaan and L. Partridge, unpublished results; (8) McCabe *et al.* (1997); (9) Robertson and Reeve (1952); (10) Tantawy *et al.* (1964); (11) Aguade *et al.* (1981).

in outbred populations may be caused by factors that are not applicable to the selection experiment reported here, such as elevated inbreeding depression in the selected lines relative to the control lines (Falconer and Mackay 1996). Furthermore, the level of asymmetry seen in the responses of outbred populations is lower than that seen in the selection response reported here or the overall asymmetrical effects of spontaneous or induced mutations seen in MA experiments. There are several possible explanations for this difference. Properties of alleles responsible for standing variation may differ from the new mutant alleles that accumulate under artificial selection or drift in MA lines. For example, deleterious pleiotropic side effects of mutations that increase size could be lower than those that decrease size, or the tails of the distributions of effects of upwardly and downwardly acting mutations could be more different than the parts of the distributions close to zero. Alternatively, the alleles responsible for selection response could be segregating at intermediate frequencies. All of these explanations imply a degree of uncoupling between mutational and standing variation.

Correlation between mutational effects on body size and fertility: Life history theory has repeatedly postulated the existence of positive pleiotropies between body size and fertility, to explain the pervasiveness of positive phenotypic and genetic correlations between the traits (Roff 1992, 2000; Stearns 1992). Such pleiotropies might arise if, for example, alleles that affect maternal body size also influence gonad size, but have rarely been tested for. The MA experiments showed a positive phenotypic correlation between body volume and fertility. Although this correlation could be caused by correlated mutational effects, Keightley *et al.* (2000) showed that, even when mutational effects are uncorrelated, strong correlations between life history traits may arise in MA lines, if different lines carry different numbers of mutations, so that the lines that carry the highest numbers of mutations are strongly affected for both traits. This possibility might account for the observation that body size and fertility were significantly correlated in the VL set only, which accumulated mutations for ~2.5 times longer than the KC set. Indeed, closer inspection of our data suggests that several MA lines did not obey the correlation: Of the lines in the top and bottom 10% for each trait in each data set, only 3 lines were simultaneously high or low for both traits, 2 lines were high for one trait and low for the other, and 30 lines

were extreme for only one of the traits (data not shown). Furthermore, body size diverged by $\sim 4\sigma_P$ in the selection experiment without a detectable correlated change in fertility. These data, taken together, suggest that mutational effects on body size and fertility are not strongly correlated. A similar conclusion was reached in a quantitative genetic study of a positive correlation between body length and fertility among recombinant inbred lines derived from a cross between the *C. elegans* isolates N2 and BO: Five distinct QTL with effects on either body length or fertility were detected, but none affected both traits simultaneously (Knight *et al.* 2001).

Mutations of large effect: The asymmetry between the overall effects of mutations increasing and decreasing size that we report here is consistent with the known distribution of mutations of large effect on growth and body size in *C. elegans*. We recently carried out a 50,000-genome EMS mutagenesis screen to search for mutations with visible body size phenotypes. Of 383 viable body size mutations isolated, 77 (20%) increased and 306 decreased some aspect of body size (Z. Z. Shen and A. M. Leroi, unpublished data). This agrees with the distribution of known loci that affect body size in *C. elegans*. The *C. elegans* database WormPD (http://www.proteome.com) lists 75 loci with deficiencies in some aspect of body size; of these, 10 (13%) increase and 65 decrease some aspect of body size (phenotypes *Lon*g *vs. Sma*ll, Short, and *Dum*py). Genome-wide RNAi screens provide a similar picture (Fraser *et al.* 2000; Maeda *et al.* 2001). The *C. elegans* database WORMBASE (http://www.wormbase.org) lists 239 loci with RNAi body size phenotypes of which 4 (2%) increase and 235 decrease some aspect of body size (phenotypes Lon *vs.* Gro, Sma, or Dpy). Since these phenotypic classifications are based largely on incidental observations rather than systematic measurements they probably underreport the number of genes that affect body size. Nevertheless all three sources suggest that mutations that increase some aspect of body size are relatively rare.

Recent studies of growth control in *C. elegans* suggest that mutations that increase body volume may be rarer than even these sources suggest. All the mutations that increase body size listed in the databases are classified as Lon, meaning they are longer than wild type. However, careful measurements of mutant alleles from several *lon* loci (*lon-1*, *-2*, and *-3*) show that while they are indeed longer than wild type, they are also thinner and so are not larger, by volume (Morita *et al.* 2002; Nyström *et al.* 2002). Similarly, while loss-of-function mutations in genes that encode components of the *C. elegans* TGF-β growth control pathway give striking dwarfism phenotypes [*e.g.*, the null allele, *dbl-1(nk3)*], worms that overexpress the TGF-β ligand, DBL-1, are not giant but merely Lon (Morita *et al.* 1999, 2002; Suzuki *et al.* 1999; Flemming *et al.* 2000; Nyström *et al.* 2002). Indeed, of the many physiological and morphogenetic processes that are required for wild-type growth in *C. elegans*, we know of only two in which deficiencies cause genuine gigantism: sensory signaling as shown by loss-of-function mutations in *egl-4* (Daniels *et al.* 2000) and a gonadal signal that represses adult growth (Patel *et al.* 2002).

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