Mutation Accumulation in Populations of Varying Size: The Distribution of Mutational Effects for Fitness Correlates in *Caenorhabditis elegans*

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ABSTRACT

The consequences of mutation for population-genetic and evolutionary processes depend on the rate and, especially, the frequency distribution of mutational effects on fitness. We sought to approximate the form of the distribution of mutational effects by conducting divergence experiments in which lines of a DNA repair-deficient strain of *Caenorhabditis elegans, msh-2,* were maintained at a range of population sizes. Assays of these lines conducted in parallel with the ancestral control suggest that the mutational variance is dominated by contributions from highly detrimental mutations. This was evidenced by the ability of all but the smallest population-size treatments to maintain relatively high levels of mean fitness even under the 100-fold increase in mutational pressure caused by knocking out the *msh-2* gene. However, we show that the mean fitness decline experienced by larger populations is actually greater than expected on the basis of our estimates of mutational parameters, which could be consistent with the existence of a common class of mutations with small individual effects. Further, comparison of the total mutation rate estimated from direct sequencing of DNA to that detected from phenotypic analyses implies the existence of a large class of evolutionarily relevant mutations with no measurable effect on laboratory fitness.

THE rate at which mutations arise and the effects that they exert on fitness are central to our understanding of a multitude of evolutionary processes. As most mutations with phenotypic effects are known to be deleterious in a given environment, their properties are key in describing the cost that recurrent mutation imposes on organismal fitness. Mutation pressure is thought to be a primary contributor to the evolution of many biological features (*e.g.,* sexual reproduction) and to be a threat to the genetic health and survival of small populations or those experiencing reduced natural selection (*e.g.,* Barton and Turelli 1989; Charlesworth and Charlesworth 1998; Kondrashov 1998; Lynch *et al.* 1999; Lynch and O'Hely 2001).

Substantial effort has been devoted to determining the contributions of the mutation rate and the average mutational effect to genetic variance, beginning with the landmark mutation-accumulation (MA) experiments conducted with *Drosophila melanogaster* by Mukai (1964) and Mukai *et al.* (1972). A high polygenic mutation rate of one per individual per generation and a low average mutational effect of <3% were suggested by these studies, indicating that conditions necessary for the accumulation of mutations via genetic drift in small populations were met. These results are widely cited and applied in many evolutionary and population-genetic models. However, while some recent studies have generally supported Mukai's findings (reviewed in Lynch and Walsh 1998 and Lynch *et al.* 1999; Eyre-Walker and Keightley 1999; Fry 2001; Fry and Heinsohn 2002), others have reported far lower deleterious mutation rates and higher average effects than those inferred by Mukai (Vassilieva *et al.* 2000; Zeyl and Devisser 2001; reviewed in Keightley and Eyre-Walker 1999 and Lynch *et al.* 1999; Chevarrías *et al.* 2001; Shaw *et al.* 2002). The studies reporting low mutation rates and large average effects include a long-term mutation-accumulation experiment conducted with the "wild-type" Bristol N2 strain of the nematode *Caenorhabditis elegans* (Vassilieva and Lynch 1999; Vassilieva *et al.* 2000). Results from this study imply that the genomic deleterious mutation rate is perhaps as much as 20 times lower and the average mutational effect twice as high as that previously measured for *D. melanogaster.* If this is a common pattern, deleterious mutation may prove insufficient to explain the diversity of evolutionary patterns in which it is currently implicated, as well as posing less of a threat to population viability.

However, the ultimate fate of mutations—loss or fixation—and the consequences for population fitness depend upon the selection coefficients (s) associated with *individual mutations.* Thus, a critical issue is the actual frequency of the most harmful class of mutations, *i.e.,* the class with high probabilities of fixation despite having

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detrimental effects on individual fitness (*e.g.*, WRIGHT 1931; GABRIEL *et al.* 1993; LANDE 1994). This group of mutations includes those with intermediate selection coefficients, which are influenced by the opposing forces of selection and drift and commonly referred to as nearly neutral or slightly deleterious (OHTA 1992). While the most damaging class of mutational effects will depend on the effective size of a population, deleterious mutations with very large or extremely small effects are unlikely to be as important for population fitness since they will be eradicated by selection or be effectively selectively neutral, respectively.

Mutations of small effect are inherently difficult to study. Nonetheless, there is growing evidence for the existence of an L-shaped or similar distribution of selection coefficients with beneficial mutations being extremely rare (reviewed in LYNCH *et al.* 1999; DAVIES *et al.* 1999; ENTIAN *et al.* 1999; WLOCH *et al.* 2001; but see SHAW *et al.* 2000, 2002). In addition, a recent study using an RNA virus demonstrated that mutations of small effect occur more frequently than those exerting large effects on fitness (BURCH and CHAO 1999). Further support for a large class of mutations with small effects comes from observations of average persistence times of mutations, measured as the ratio of standing genetic variation to new mutational variance, which imply average selection coefficients for heterozygous mutations of only 1–2% (reviewed in HOULE *et al.* 1996 and LYNCH and WALSH 1998; LYNCH *et al.* 1998).

Empirical evaluation of the relationship between population size and mutation accumulation should provide basic information on the spectrum of fitness effects of new mutations and thus potentially lend insight into the above dichotomy. A possible means of discerning the most critical portion of the distribution of selection coefficients would be equivalent to asking how large a population must be before it behaves genetically as though it were effectively infinite in size. To achieve this, we conducted a set of divergence experiments in which parallel MA lines of *C. elegans* were maintained in a range of different population sizes. The underlying concept of this approach is that, by increasing the bottleneck size, the efficiency of selection against mutations of larger effect will be generally improved and the range of selection coefficients of accumulated mutations will be restricted into successively narrower classes. A significant fitness reduction in large-population lines would then imply the existence of a common class of mutations with small effects.

Because spontaneous mutation causes a rate of fitness reduction of <1%/generation in the N2 lines maintained at $N = 1$ (hereafter referred to as "natural MA lines"; VASSILIEVA *et al.* 2000), we wished to use a strain with an elevated rate of mutation for this study. For these purposes, we obtained a DNA mismatch repair (MMR)-deficient strain of *C. elegans*, *msh-2*. In addition to our evaluation of the sensitivity of this line to various

levels of random genetic drift, the analysis of lines maintained at $N = 1$ provides the first estimates of the genomic mutation rate and average mutational effect in the absence of mismatch repair for this species. This is an issue with potential impact for human health, as defects in Msh2 are known to be associated with increased risk of developing certain forms of cancer in mammals (reviewed in BUERMEYER *et al.* 1999 and HARFE and JINKS-ROBERTSON 2000).

Msh2 is a homolog of the *Escherichia coli* gene, MutS, and its product is known to be a critical component of all forms of DNA mismatch repair in other eukaryotes (*e.g.*, HARFE and JINKS-ROBERTSON 2000). Consequently, *msh2* null mutants are expected to lack MMR completely. DEGTYAREVA *et al.* (2002) showed that MSH-2 indeed plays a role in DNA repair in *C. elegans*, finding elevated microsatellite instability and increased reversion rates, as well as reduced survivorship and fertility, in lines of this *msh-2* strain. On the basis of their results, we anticipated that the mutation rate in this line would increase ~100-fold for microsatellite changes over that seen in the natural MA lines (VASSILIEVA *et al.* 2000), which would be roughly consistent with results of previous studies examining the consequences of knockouts of MutS in *E. coli*, and Msh2 in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, *D. melanogaster*, and mouse (REENAN and KOLODNER 1992; STRAND *et al.* 1993; JOHNSON *et al.* 1996; MARISISCHKY *et al.* 1996; SIA *et al.* 1997; WEIRDL *et al.* 1997; BUERMEYER *et al.* 1999; FLORES and ENGELS 1999; HARFE and JINKS-ROBERTSON 2000; MANSOUR *et al.* 2001; ZEYL and DEVISSER 2001). Each of these studies found 100- to 1000-fold increases above wild-type levels in both microsatellite instability and single-nucleotide substitutions.

MATERIALS AND METHODS

Base strain: The experiments reported here were conducted using a strain of *C. elegans* with a Tc1 transposon inserted into the reading frame of the Msh2 gene (H26D21.2). The mutant was isolated from strain MT-3126 in a PCR-based screen by the T. D. Petes laboratory (University of North Carolina, Chapel Hill, NC) and was repeatedly backcrossed (10 times) to the N2 strain, tracking the *msh-2(e679::TC1)* allele by PCR (see DEGTYAREVA *et al.* 2002 for details). This procedure produced a strain with minimal residual levels of transposition, within which the *msh-2* allele occurred in a nearly entirely N2 genetic background. Prior to this experiment, the strain was further backcrossed to N2 and inbred by self-fertilization for 10 generations to achieve homozygosity. Subsequently, many thousands of these animals were frozen for use as a control (LEWIS and FLEMMING 1995). Not surprisingly, our *msh-2* base strain exhibited diminished performance compared with the repair-proficient N2 strain (VASSILIEVA and LYNCH 1999; VASSILIEVA *et al.* 2000) at the initiation of these experiments, presumably as a consequence of the joint effects of a high mutation rate and relaxed natural selection experienced during serial passage.

Experimental line generation and maintenance: We conducted two separate divergence experiments. The first was

initiated with three sets of 50 lines of *C. elegans*, all derived from the offspring of a single, highly inbred *msh-2* hermaphrodite. Each set of lines was propagated by self-fertilization at a different population size—1, 5, and 25 individuals—for 10 generations. We chose bottleneck sizes for the first experiment that would be expected to capture mutations having a fairly broad array of selection coefficients. The dynamics of mutations with effects $\ll 1/(2N_e)$ are expected to be governed exclusively by random genetic drift, causing them to accumulate at close to the neutral rate (KIMURA 1983, Chap. 3). Under complete selfing, $N_e = 1$, so populations of the above sizes would be expected to accrue mutations with selection coefficients $< \sim 0.50$, 0.10, and 0.02, respectively. The borders between the mutational classes for each population-size treatment are rather arbitrary, however, as the probability of eventual fixation of a mutant allele declines continuously over a fairly broad range of values of $4N_e s$ (KIMURA 1983, Figure 3.7). As outlined below, we therefore use a transition-matrix approach that allows exact calculations of fixation probabilities of deleterious mutations in selfing populations of differing size and subsequent estimates of average fitness decline expected in such populations.

All lines were maintained under standard laboratory conditions (at 20° on 60- × 15-mm petri plates with nematode growth medium agar uniformly seeded with a suspension of OP50 *E. coli*; SULSTON and HODGKIN 1988). As detailed in VASSILIEVA and LYNCH (1999), experimental lines were maintained by transferring L4-stage offspring produced near the middle of the reproductive period. The method of transferring immature hermaphrodites each generation ensured that populations maintained at larger bottleneck sizes experienced no sexual reproduction. Thus, the larger populations should not have experienced reduced deleterious mutation accumulation or an increased rate of fixation of beneficial alleles due to sexual recombination.

On the basis of results of the first assay, we chose different population sizes ($N = 1$, 2, 3, and 10 individuals) for the second assay, allowing us to examine the pattern of fitness reduction on a finer scale. Sets of 40 lines were maintained at these population sizes for 20 generations prior to being assayed for fitness-related characters. We decided to extend the number of generations for the second experiment so that, if mutations with the sufficiently low selection coefficients arose, there would be time for a greater number to accumulate in the populations of larger size. These lines were generated from the same control population as those from the first experiment. However, the control was less fit in the second assay, presumably due to the additional bottlenecks experienced after thawing prior to this experiment.

To prevent accidental line loss, lines that went extinct over the course of the experiments were reinitiated from populations of the previous generation as many as five times prior to being considered extinct in the experiment. Such line extinction was observed only in the lines maintained in single-individual bottlenecks. Consequently, this study disregards lethal mutations and therefore certainly underestimates the total deleterious mutation rate.

Fitness assay procedure: Assays including survival to maturity, progeny production, and intrinsic rate of population increase were conducted on single individuals in a benign environment after 10 generations of divergence for the first experiment and after 20 generations for the second experiment. These assays were conducted in parallel with the frozen ancestral (*msh2*) control. The assay procedure and methods for estimation of survival and productivity are detailed in VASSILIEVA and LYNCH (1999). Briefly, prior to each assay, all lines were expanded into five replicates (four replicates for the second assay) and maintained by transferring single indi-

viduals for two generations. Fitness components were then measured using single, third-generation individuals from each replicate. The same general procedure of line subdivision was applied to 20 thawed control animals. Survival to maturity was scored as 1 if an animal survived from the first larval stage to produce a surviving offspring, and otherwise as 0. Single individuals were transferred to fresh plates daily and progeny production was measured by directly counting the progeny produced over the first 4 days of life, covering the majority of the reproductive period. Intrinsic population growth rate was calculated for each line by solving $\Sigma e^{-rx} l(x) m(x) = 1$ for r, where $l(x)$ is the proportion of worms surviving to day x and $m(x)$ is the fecundity at day x.

Differences among the means for each character in each population-size treatment were assessed using Bonferonni *t*-tests. All pairwise comparisons were performed for each of the two experiments using SPSS 11.0 statistical software (SPSS 2001).

Estimation of mutational parameters: The deleterious mutation rate and average mutational effect for each population-size treatment were estimated using two different methods. The Bateman-Mukai (B-M) method (BATEMAN 1959; MUKAI 1964; MUKAI *et al.* 1972), described in detail in VASSILIEVA and LYNCH (1999), relates changes in the mean and among-line variance of a character experiencing mutation to the genome-wide mutation rate and average mutation effect. For both controls and MA lines ($N = 1$ population size treatment), individual nested analyses of variance were performed to obtain within- and among-line variance components for each trait, which provide estimates of the environmental and genetic variance, respectively. Assuming an approximately linear decline in fitness with time (a common characteristic of MA experimental results), the rates of change in the mean (R_m) and among-line variances (V_b) for MA lines were computed for each trait. Point estimates for U_{min}, the minimum diploid genomic mutation rate for a character, and a_{max}, the maximum average homozygous mutational effect, were made for each trait, using the formulas $U_{min} = 2R_m^2/V_b$ and $a_{max} = V_b/R_m$. Dividing a_{max} by the control mean phenotype yields a measure of the proportional reduction in the trait per homozygous mutation. These estimates of U_{min} (a_{max}) may be downwardly (upwardly) biased as they disregard variation in selection coefficients among new mutations.

A bootstrapping program, H2BOOT (PHILLIPS 2002), was used to obtain estimates of U_{min}, a_{max}, the mutational heritability (h_m^2), and their 95% confidence intervals. The mutational heritability was estimated for each trait as the ratio of the mutational variance, taken to be half the rate of increase in among-line variance, and the environmental variance, equivalent to the within-line variance component for a trait. Bootstrap estimates of U_{min} and a_{max} are based on the B-M technique described above; but we report the average of the bootstrap estimates, which should be less biased than the standard parametric estimates (EFRON and TIBSHIRANI 1993). We generated 10,000 bootstrap estimates (with replacement) of each parameter and estimated the 95% confidence intervals directly from frequency distributions of the estimates.

To obtain potentially less biased estimates of the genomic mutation rate and average effect for productivity and intrinsic population growth rate, we included a maximum-likelihood (ML) procedure described in detail in VASSILIEVA *et al.* (2000). Although ML makes fuller use of the data, unlike the above methods, it must make explicit assumptions regarding the distributions of both mutational and environmental effects. The procedure arrives at values for the mutation rate and average effect by evaluating the log-likelihood of the mean of each MA line as a function of specified parameter combinations. Final estimates are made such that the likelihood of the total pool of data is maximized. Briefly, the procedure assumes

that mutations are distributed among lines in a Poisson manner with expectation $Ut/2$, where U is the diploid genomic mutation rate and t is the number of generations of divergence. As experienced in the study involving the natural MA lines (Vassilieva *et al.* 2000), the distributions of residual errors for both characters were nonnormal, as noted above. A key feature of our ML procedure, however, is that the distribution of residual errors is described using the empirical distribution of deviations of individual measures from control means standardized by the within-assay standard deviation. This aspect distinguishes our approach from that developed by Keightley (1994, 1996), which assumes normally distributed residuals. Approximate 95% confidence intervals for each estimate were obtained by identifying the combination of U and a at which the log-likelihood score for U was 2 units lower than the maximum.

We performed ML analyses assuming constant mutational effects (as with the B-M estimators) for productivity and r for the $N = 1$ population-size treatments in both assays. We also attempted to perform analyses allowing variable mutational effects (assuming gamma-distributed mutational effects) for these traits. Likelihood analyses involving variable effects are essentially identical to those for the constant-effects model with the addition of a third component, the coefficient of variation of mutant effects (see Vassilieva *et al.* 2000 for details).

Observed *vs.* expected fitness decline: We wished to explore the possibility that mutations of small effect were reaching fixation in our experimental lines. To evaluate whether the mean fitness of lines maintained at different population sizes was in accord with expectations based on the fixation of mutations with the assumed (extremely large) constant effect size, we compared the decline in fitness shown by each population-size treatment to that expected on the basis of our B-M estimates of mutation rate and average (constant) mutational effects for productivity and r made using the $N = 1$ lines. As in Lynch *et al.* (1995), we used a transition matrix approach to obtain the expected *cumulative* reduction in fitness due to fixations of new mutations—a function of the number of mutations entering the population per generation, the probability of fixation of those mutations, and the reduction in population fitness per fixation. To account for the self-fertilizing nature of *C. elegans*, we followed the method of Schoen *et al.* (1998) to compute the changes in the genotypic (rather than allelic) composition of populations of different size over time for new mutations experiencing selection and genetic drift.

For a population of size N, the probability of having a particular genotypic composition in the subsequent generation, given that in the present generation, was obtained by Equation 2 in Schoen *et al.* (1998), into which our B-M estimates for average mutational effect, s, were inserted. The resulting transition probabilities form the elements of a $[(N + 1)(N + 2)/2 \times (N + 1)(N + 2)/2]$ matrix, **P**. The expected number of loci in each of the genotypic states at time $t = 1$ is $\mathbf{g}_{t=1} = \mathbf{P}\mathbf{g}_{t=0}$, where **g** denotes a column vector containing elements equal to the expected numbers of loci in each of the $(N + 1)(N + 2)/2$ possible states, ranging from loss ($i = 0$) to fixation ($i = 2N$) of the mutant allele. This equation was iterated 10 or 20 times, with UN mutations entering the population per iteration, to give the state of the population after an equivalent number of generations (to generate relevant results for both the 10- and 20-generation experiments). The mean fitness of the population resulting from all new fixations during this time course was obtained from Equation 13 in Lynch *et al.* (1995).

Each population was initiated with mutation-free individuals and offspring acquired new mutations prior to selection. Note that this life cycle sequence differs from that in Schoen *et al.* (1998), where selection operated on adults prior to segregation. All new mutations were assumed to arise in single copies at loci not currently segregating in the population, have additive effects on fitness, and have independent evolutionary trajectories.

Molecular analyses: To understand the total mutational pressure experienced as a result of the MSH-2 knockout, we conducted a molecular survey of the 38 surviving $N = 1$ lines from the second divergence experiment. A subset of homopolymer loci (Denver *et al.* 2004) as well as complex nuclear sequence (*i.e.*, nonrepetitive sequence) previously analyzed in the natural MA lines (D. Denver, K. Morris, M. Lynch and K. Thomas, unpublished results) was examined for these sets of repair-deficient lines. We directly sequenced >9 kb of nuclear DNA, representing all five autosomes and the X chromosome, for each MA line and the ancestral control. As the *msh-2* mutation was backcrossed into the N2 background, comparison to sequence results from the natural MA lines allows a direct assessment of the increase in molecular mutation rate and change in pattern of effects resulting from this mutational pathway.

In addition, a separate set of 45 *msh-2* lines also propagated at $N = 1$ for 20 generations from another study (D. Denver, S. Estes, K. Thomas and M. Lynch, unpublished results) was surveyed for molecular variation by sequencing 20 kb of nuclear DNA—again, a subset of the complex sequence examined in the natural MA lines.

RESULTS

Effect of *msh-2* and population size on fitness correlates: The *msh-2* strain exhibited a lower baseline level of fitness than its repair-proficient progenitor, N2, at the outset of the experiment. In addition to having a longer generation time, the repair-deficient control exhibited values for average productivity, survival, and intrinsic rate of increase that were depressed by ~36, 9, and 39%, respectively, compared with the average performance of our N2 control (Vassilieva *et al.* 2000). Four N2 control lines with five replicates each (*i.e.*, 20 individuals) were included in the second assay and shown to perform at ordinary levels for each trait (data not shown). As this suggests that our current experimental results were at least not greatly influenced by environmental fluctuations in the laboratory, we are comfortable making rough comparisons of fitness data from this experiment with those from the previous N2 studies (Vassilieva and Lynch 1999; Vassilieva *et al.* 2000).

Population size was extremely efficient in ameliorating fitness decline, its effect highly significant across all traits and population-size treatments in both assays. For example, Figure 1 relates the influence of population size on the variation among line means for intrinsic growth rate for each population-size treatment (data from a 20-generation experiment), showing a dramatic downward shift in the distribution of mean intrinsic growth rates as population size decreased. A similar pattern was observed for survival and productivity in both experiments. There was also a significant effect of line within treatment for all tests ($P < 0.0001$), indicating that replicate lines within population-size treatments
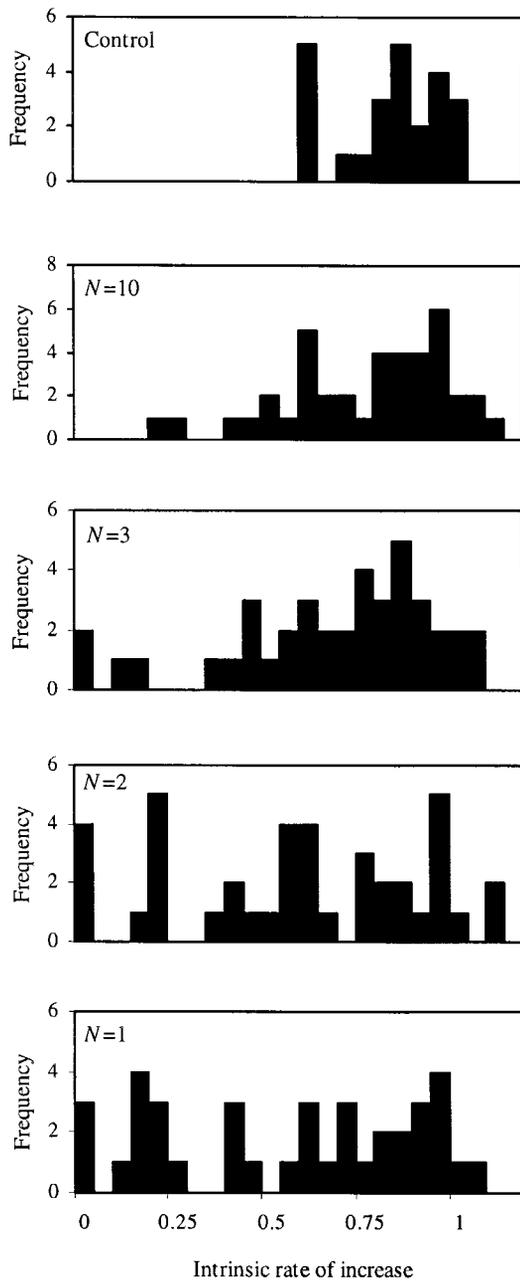
FIGURE 1.—Variance among lines. Shown are the distributions of mean intrinsic rate of increase for lines in each population-size treatment after 20 generations of mutation accumulation compared to the distribution of 20 *msh-2* control lines (top). The control lines provide an estimate of the among-line environmental variance, while the increase in variance beyond this shown by the MA lines lends insight into the amount of variation produced by mutation.
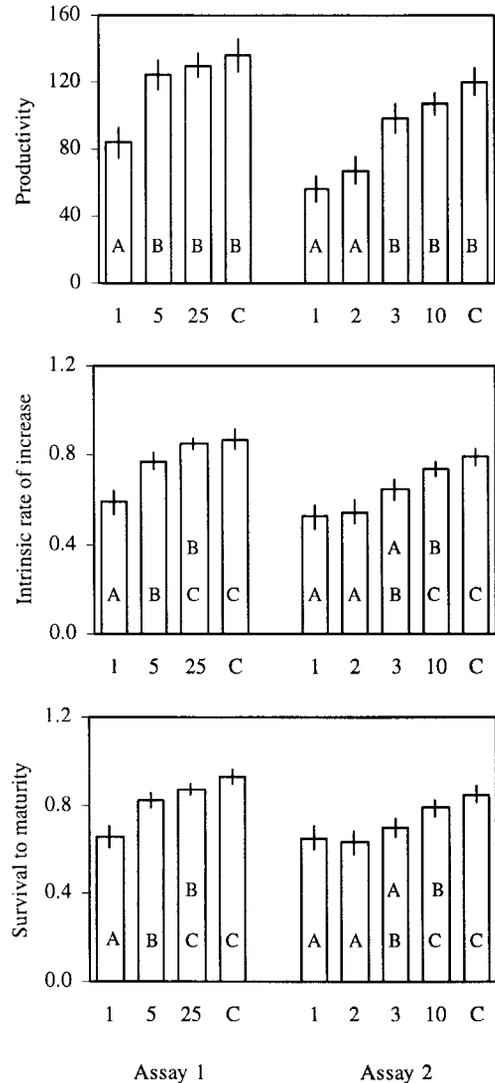

FIGURE 2.—Trait means for different population-size treatments in both assays. Each bar represents the mean phenotype for a particular population-size treatment, listed on the *x*-axis with the control (C). Results for the 10-generation assay (Assay 1) are on the left side of each panel, while those for the 20-generation assay (Assay 2) are on the right. Error bars represent one standard error. For each set of bars, groups of means labeled with different letters indicate a significant difference at the $\alpha = 0.05$ level (Bonferonni *t*).

had diverged substantially from one another with respect to these traits.

Figure 2 shows trait means for each bottleneck-size treatment in both the 10-generation (assay 1) and 20-generation (assay 2) experiments. The pattern of increasing character means with increasing population size is readily evident. Notably, the frequency of mutations causing visible phenotypes (*e.g.*, roller, uncoordi-

nated) in the different population-size treatments was qualitatively similar to the pattern of fitness decline with population size. In the $N = 1$ treatment, discernible morphological mutations arose and were fixed at a rate of 0.0038/generation, while for the $N = 2$ lines, the rate of fixation was 0.0025/generation; no such phenotypes were observed in the larger population-size treatments. Comparatively, the rate to such visible mutations observed in the natural MA lines is >20 times lower at 1.3×10^{-4}/generation (S. ESTES and M. LYNCH, unpublished data).

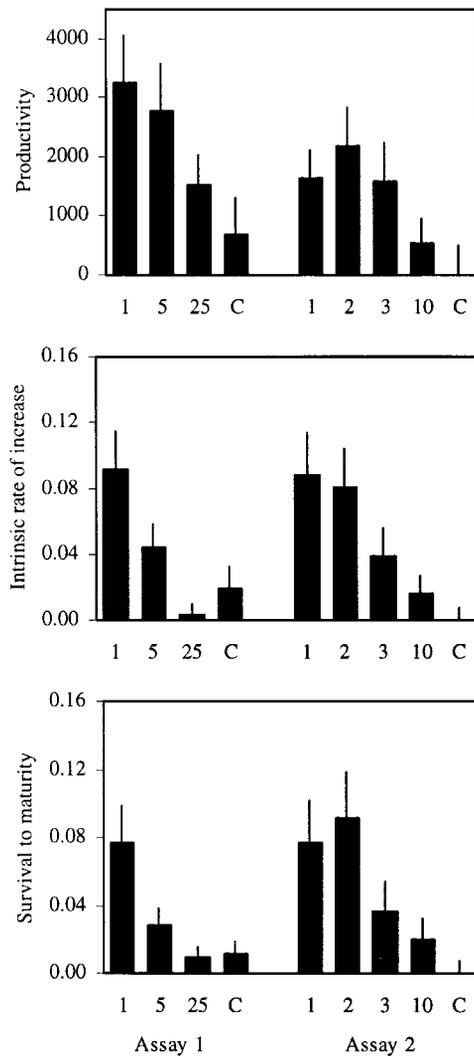The decrease in fitness-related trait means with decreasing population size was accompanied by an in-

FIGURE 3.—Patterns of among-line variance for each trait. Each bar represents the variance among lines within a particular population-size treatment, listed on the *x*-axis with the control (C). Results for the 10-generation assay (Assay 1) are on the left side of each panel, while those for the 20-generation assay (Assay 2) are on the right. Error bars represent one standard error.

crease in among-line variance for all characters, conforming to the expected pattern of a reduction in genetic variance as the effects of selection counter those of drift (Figure 3). Controls exhibited nonsignificant among-line variance for all traits in both assays.

Genetic mutation parameters: The lines maintained at $N = 1$ allow us to estimate, on a per character basis, the change in the genome-wide rate (U) and average effect (a) of mutations as a result of *msh2*. Results of each method of parameter estimation for the $N = 1$ lines in the second experiment (*i.e.*, after 20 generations of single-individual bottlenecks) are reported in Table 1 with those from the natural MA lines (VASSILIEVA *et al.* 2000) for comparison. The different methods of estimation yield fairly similar results for both parameters. Taken together, all methods of estimation indicate

that the average rate of mutation for fitness-related characters is increased in this strain by a factor of $\sim$10 over that of the natural MA lines or by factors of 9, 4, and 18 for productivity, *r*, and survival, respectively. The average effects of mutations generated in the current experiment were fairly similar to those estimated for the natural MA lines, except that estimated for *r*, which was approximately twice as high as the average effect estimated for the natural MA lines.

Both the B-M and ML methods of estimating mutational parameters assume unidirectional (*i.e.*, consistently detrimental) mutation effects. As can be seen in Figure 1, a few lines in each of the population-size treatments showed higher performance than the control. Using two standard errors as a criterion for statistical significance, 1, 2, 2, and 3 out of 40 lines exhibited mean values for *r* significantly higher than the control mean in the population-size treatments of 1, 2, 3, and 10, respectively. (Other traits showed a similar pattern.) As there were twice as many lines as control lines in each population-size treatment, much of this pattern is probably due to sampling effects. Yet, this may indicate that a slight fraction of incoming mutations produced fitness-enhancing effects. Although the assumption of unidirectional mutation effects may be very mildly violated in our experiment, potentially resulting in an underestimate of the total mutation rate (SHAW *et al.* 2002), this effect is probably slight in our study and does not affect the main conclusions drawn here. Notably, no lines in the long-term natural MA experiment have ever been observed to significantly improve beyond the control with respect to the typically measured fitness-related characters.

The true genomic mutation rate for *msh2* may be higher than what we are able to detect here due to variation in mutational effects. We attempted to perform ML analyses allowing variable mutational effects for productivity and *r*. However, our analyses indicate either that the variable-effects model did not fit significantly better than the constant-effects model or, more likely, that the model could not be adequately fit to the data due to an inadequate number of controls coupled with a fairly high frequency of control replicates with no reproductive output. These results are therefore not presented here.

Observed *vs.* expected fitness decline in populations of different size: Although not statistically significant, our largest population sizes (*i.e.*, 10 and 25) consistently exhibited slight reductions for all three fitness-related phenotypes compared to control means in both experiments (Figure 2). Under a scenario in which all incoming mutations produce effects on fitness equal to our B-M estimates of average effect (Table 1; *i.e.*, under a model of constant, large mutation effects), our calculations (see MATERIALS AND METHODS) indicate that no decline in productivity or *r* due to fixations of new mutations would be expected in these larger populations

TABLE 1

Parameters for mutations affecting fitness correlates

Strain	Method	U	a	h_{m}^2
		Productivity		
msh-2	B-M	0.249	−0.211	0.0168 (0.0047)
	Boot	0.274 (0.496, 0.144)	−0.106 (−0.062, −0.155)	
	ML_C	0.383 (0.696, 0.158)	−0.160 (−0.100, −0.342)	
N2	B-M	0.049 (0.024)	−0.088 (0.032)	0.0008 (0.0003)
	ML_C	0.018 (0.010, 0.037)	−0.369 (−0.446, −0.305)	
		r		
msh-2	B-M	0.081	−0.416	0.0213 (0.0071)
	Boot	0.090 (0.178, 0.036)	−0.211 (−0.121, −0.322)	
	ML_C	0.069 (0.145, 0.044)	−0.529 (−0.281, −0.528)	
N2	B-M	0.014 (0.006)	−0.222 (0.060)	0.0033 (0.0008)
	ML_V	0.024 (0.012, 0.259)	−0.131 (−0.219, −0.033)	
		Survival		
msh-2	B-M	0.051	−0.458	0.0127 (0.0051)
	Boot	0.058 (0.124, 0.018)	−0.232 (−0.115, −0.386)	
N2	B-M	0.003 (0.001)	−0.390 (0.084)	0.0031 (0.0007)

Estimates of the per generation genomic mutation rate (U), average mutational effect (a), and mutational heritability (h_{m}^2) for each trait for the $N = 1$ population-size treatment in the 20-generation experiment compared to those for the natural MA lines (N2) are shown. B-M point estimates, bootstrapped B-M estimates (Boot), and ML estimates (ML_C and ML_V are for constant and variable-effects models, respectively) of U and a are also shown. B-M and ML (averaged across five assays) estimates for the natural MA lines (N2) are taken from Vassilieva *et al.* (2000, Tables 3 and 4). Only estimates from the ML model that provided the best fit are listed for the natural MA lines. One standard error or 95% confidence intervals are shown in parentheses following each estimate.

(Figure 4). This is because once the effective population size reaches 10–15 individuals selection could effectively purge such large-effect mutations. (The binomial nature of survivorship causes the estimates for this trait to be quite noisy; it was thus omitted from the analysis.) The fact that fitness does decline more than expected in larger populations (Figure 4) may indicate that either (1) small-effect mutations are managing to reach fixation and cause fitness reduction in the larger populations or (2) this "extra" decline is due to segregation load, *i.e.*, due to mutations of unknown effect size segregating in our large-population lines.

Change in per nucleotide mutation rate: Analyses of mutations detected from direct sequencing of experimental line DNA confirm that the effect of a *msh-2* knockout is to increase the total mutation rate. As expected, the mutational patterns that we observed were similar between both sets of experimental lines sequenced [*i.e.*, between the 40 $N = 1$ lines used in the phenotypic study reported here and the 45 additional such lines from a separate study (D. Denver, S. Estes, K. Thomas and M. Lynch, unpublished results)]. We estimate the molecular mutation rate (SE) in complex sequence to be 2.2×10^{-6} (3.5×10^{-7})/nucleotide/ generation, an ∼100-fold increase over the estimate of 2.3×10^{-8} (6.0×10^{-9}) previously measured in natural *C. elegans* MA lines (D. Denver, K. Morris, M. Lynch

and K. Thomas, unpublished results). For homopolymeric runs, we estimate the locus-specific mutation rate to be 5.3×10^{-2} (8.3×10^{-3})/generation compared with 3.0×10^{-4} (1.2×10^{-4})/generation measured for homopolymer sequences with comparable motif size in the natural lines loci (Denver *et al.* 2004), an almost 180-fold increase.

DISCUSSION

Resolution of a number of difficult problems in evolutionary and conservation genetics awaits accurate estimates of the magnitude of individual mutational effects. For example, expectations for the response of populations to long-term selection, the evolution and maintenance of sexual reproduction, the probability of long-term genetic health in human populations, and the level of bias involved in estimating the underlying components of mutational variance—the genomic mutation rate and the mutational effects—rely on the degree of variation in mutational effects (*e.g.*, Crow and Kimura 1970; Lande 1976; Crow and Simmons 1983; Barton and Turelli 1989; Kondrashov 1995; Gessler and Xu 1999; Lynch *et al.* 1999; Poon and Otto 2000). Also, the main approach taken for determining critical effective sizes beyond which the accumulation of genetic damage is unlikely is rooted in models of the mainte-
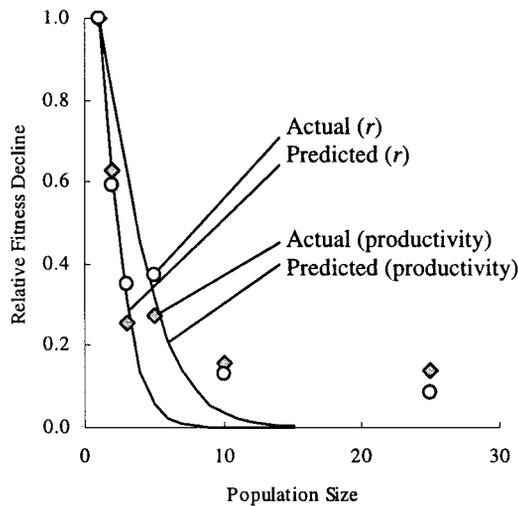
Figure 4.—Observed *vs.* predicted relative fitness decline. Open circles (shaded diamonds) represent the average decline in intrinsic rate of population increase (progeny production) for each population size relative to that shown by the $N = 1$ population size treatment. Relative decline was calculated as the rate of change in mean phenotype relative to the control for each population size treatment divided by that shown by the $N = 1$ population size treatment. Solid curves represent the relative decline for each phenotype expected on the basis of Bateman-Mukai estimates of average mutational effect for each character.

nance and fixation of genetic variation in populations through a balance among mutation, genetic drift, and/or natural selection (Lande 1976; Franklin 1980; Turelli 1984; Lynch and Lande 1998; Bürger 2000, Chap. VII). As the spectrum of new mutational effects can impact the amount of equilibrium additive genetic variation maintained in populations, the shape of this distribution could have significant effects on the recommendations of such models (*e.g.*, Lynch *et al.* 1998; Whitlock *et al.* 2003).

To assess the consequences of reducing the effective population size for the efficiency of selection against newly arisen mutations, we conducted a series of divergence experiments in which sets of self-fertilizing lines of the *msh2* strain of *C. elegans* were maintained at a range of population densities. The dynamics of mutations with selection coefficients much less than the reciprocal of the effective population size $[|s| \ll 1/(2N_e)]$ are expected to be entirely governed by random genetic drift. In standard MA experiments involving selfing species, $N_e = 1$, so all mutations with effects $< \sim 50\%$ are expected to contribute to the mutational degradation. By increasing the population size and allowing natural selection to occur, we expect that mutations reaching fixation in our larger population-size lines will have proportionately smaller average effects.

We also utilize previous work on the properties of spontaneous mutation (Vassilieva and Lynch 1999; Vassilieva *et al.* 2000) and on molecular evolution

(Shabalina and Kondrashov 1999; Denver *et al.* 2000; D. Denver, K. Morris, M. Lynch and K. Thomas, unpublished results) in *C. elegans* to directly assess the effect of *msh2* at the molecular level and to make inferences regarding the distribution of mutational effects on fitness.

Effect of *msh-2* on mutation parameter estimates: The impact of *msh-2* on the baseline deleterious mutation rate as well as estimates of the average mutational effect were assessed from the results of the $N = 1$ treatments. As expected, the *msh-2* strain exhibited a "mutator" phenotype with the overall rate of detectable mutations on lab fitness increasing from the natural MA line average of 0.019 to 0.146 mutations/character/generation. The average mutational heritability (SE), 0.0169 (0.006), is increased by a similar proportion, approximately sevenfold over that calculated from the natural MA experiment. Note that even though the per character deleterious mutation rate is increased in the *msh-2* strain over that measured in the natural MA lines, it is still low relative to estimates from other organisms (*e.g.*, Drosophila and Daphnia), while our estimates of mutational heritability are approximately six times higher (Lynch and Walsh 1998). This implies a higher rate of change in the variance among our *msh-2* lines of *C. elegans* relative to the rate of change in the mean phenotype and to the environmental (within-line) variance, respectively, compared to that observed in other organisms. Our estimates for the average effect of mutations influencing productivity and survival were similar to those computed for the natural MA lines, suggesting that the spectrum of effects caused by this mutational pathway is not drastically different from that caused by all spontaneous mutations for these traits. However, estimates of average mutational effect size for mutations influencing population growth rate were approximately twice as high as those inferred from the natural MA. Unlike a recent study with *msh2* yeast (Zeyl and Devisser 2001), we did not observe a reduction in average mutational effect size for this *msh-2* strain.

Effect of population size for fitness decline and the distribution of mutational effects: The signature of selection was evident in patterns of change in the character means and among-line variances with increasing population size. One of the most striking results of this study is that, even under the increased mutational pressure caused by the absence of DNA repair, a very small effective population size was apparently sufficient to prevent accumulation of most new mutations with measurable effects on fitness on a timescale of 20 generations. The decline in fitness with decreasing population size suggests that the majority of the mutational variance discernible in the benign environment of the lab is due to mutations producing large, negative effects as population sizes of three were able to maintain fairly high fitness levels relative to the control over the course of the study. This result is congruent with our estimates

of average mutational effects (Table 1). (The fact that the *msh2* strain began the experiment with fitness well below the N2 control may place a bound on how large a population-size effect could be detected in this experiment.)

However, even the largest population-size treatments show a slight trend toward reduced fitness compared to the control. Our comparisons of observed *vs.* expected declines in fitness correlates (Figure 4) show that fitness reduction observed in the larger-population-size treatments was greater than that expected on the basis of our B-M estimates of average mutation effect, while the decline observed in smaller population sizes closely matched the expectations. There are at least two possible explanations for this observation: (1) a fraction of small-effect mutations managed to reach fixation in the large population lines or (2) the reduced fitness at the larger population sizes is due to still-segregating mutations that arose during the experiment. We are unable to distinguish between these two possibilities with our data, but as it takes an average of $4N_e$ generations for a neutral mutation to reach fixation, the latter scenario seems more likely since mutations may not have had sufficient time to reach fixation in the larger populations. Additionally, in light of the extremely large effects of incoming mutations (Table 1), it seems probable that mutations with such detrimental effects would have a transiently negative impact on the fitness of large-population-size lines. On the other hand, time to fixation for a selected mutation (either beneficial or deleterious) can be much shorter than $4N_e$ generations depending on dominance relationships and $|Ns|$ (Ewens 1979; Bürger 2000, Appendix), so it remains feasible that fitness reduction in our large-population-size lines is at least partly a result of actual fixations of deleterious alleles. This quandary underscores the need for the development of multilocus theory for evaluating expected fitness decline due to both segregation and fixation loads in obligate selfers—a difficult problem that has thus far received little attention from theoreticians.

Alternatively, the overall pattern of fitness decline across the different population sizes (Figure 4) could be compatible with the existence of diminishing returns epistasis, which causes the attenuation of mutational effects with the acquisition of additional mutations. Because we observed a greater-than-expected decline in the larger-population-size treatments (containing fewer mutations), this could indicate that the composite effect of mutations in the small-population-size lines (containing relatively more mutations) was dampened by such a nonadditive genetic effect. Unfortunately, fitness decline was sufficiently rapid in our $N = 1$ lines that we did not conduct multiple assays and thus can make no further statements about the pattern of fitness decline or potential existence of net epistasis among new variants.

Observed *vs.* predicted mutation rates for fitness: Our results indicate a large discrepancy between mutation rates estimated from phenotypic and molecular analyses of the *msh2* $N = 1$ lines. This is our most compelling evidence for a class of mutations with small selective effects.

The molecular mutation rate for complex sequence in the *msh2* strain was estimated to be almost 100 times higher than that in the N2 strain and almost 200 times higher than that for homopolymer loci. These estimates are consistent with our expectations drawn from other studies of Msh2 knockouts, including the smaller-scale survey of microsatellite instability and reversion rates of a visible mutation conducted by Degtyareva *et al.* (2002), using the same strain we employed here. In light of recent evidence for a substantial class of mutations having cryptic effects on laboratory fitness (Davies *et al.* 1999), we explored this issue by simple comparison of the expected number of mutations per line predicted from the molecular mutation rate to that inferred from phenotypic analyses. Using the bootstrap estimates for the rate of mutations affecting fitness characters (Table 1), the expected number of mutations per *msh2* line ($N = 1$ treatments) affecting each character after 20 generations is 2.74 for productivity, 0.58 for survival, and 0.90 for intrinsic rate of increase. We can approximate the expected mean number of total mutations per line using our estimate of per nucleotide mutation rate in complex sequence, 2.2×10^{-6} (3.5×10^{-7})/generation. Coding DNA constitutes ~27% of the *C. elegans* genome, or ~26.2×10^6 bp (*C. elegans* Sequencing Consortium 1998). Disregarding codon bias (making our estimate more conservative) and assuming all types of substitutions are equally probable, 75% of all possible nucleotide substitutions are expected to cause an amino acid change (Li 1997, Table 1.4). Using these parameters, we expect an average of ~864 nonsynonymous mutations per ($N = 1$) line after 20 generations (*i.e.*, $2.2 \times 10^{-6} \times 26.2 \times 10^6$ bp $\times$ 20 generations $\times$ 0.75). Although this is a rough estimate, it is apparent that only a small fraction, perhaps $\leqslant 1\%$, of the mutations in complex sequence have detectable effects on laboratory fitness. Additionally, by including these mutations with neutral effects on laboratory performance we can compute an adjusted estimate of the average effect size of mutations in the benign assay environment. If the true per generation genomic mutation rate is ~43 as suggested by the molecular analyses (864 mutations/20 generations), the average homozygous mutational effect compatible with the observed variance among $N = 1$ lines for progeny production, for example (Figure 1), is -0.016, an order of magnitude lower than what we estimate from the phenotypic analyses (Table 1). This follows from the expected increase in among-line variance being equal to $2UE(a^2)$, where U is the diploid genomic mutation rate for a character and $E(a^2)$ is the average squared effect (Vassilieva and Lynch 1999).

Obviously, extremely small *individual* mutational ef-

fects cannot be detected in a finite experiment, but if such mutations occur frequently, their cumulative effects should be detected in an experiment of this kind. The above result is fairly consistent with those of DAVIES *et al.* (1999) where it was estimated that 96% of all EMS-induced mutations were undetectable in standard fitness assays in a benign environment. Coupled with evidence for the action of strong purifying selection on protein-coding and regulatory sequence (SHABALINA and KONDRASHOV 1999; DENVER *et al.* 2000; WEBB *et al.* 2002), it is likely that typical mutation-accumulation experiments vastly underestimate the rate of occurrence of mutations that would be detrimental in nature. This underestimate is not due to an inherent limitation of MA experiments, but instead is likely to be caused by the laboratory serving as a poor substitute for natural environments. Taken together, our results underscore the critical need for future studies addressing the potential environmental influence on the spectrum of mutational effects and its impact on population fitness.

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