A Test for Epistasis Among Induced Mutations in *Caenorhabditis elegans*

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ABSTRACT

Synergistic epistasis, in which deleterious mutations tend to magnify each other's effects, is a necessary component of the mutational deterministic hypothesis for the maintenance of sexual production. We tested for epistasis for life-history traits in the soil nematode *Caenorhabditis elegans* by inducing mutations in two genetic backgrounds: a wild-type strain and a set of genetically loaded lines that contain large numbers of independent mildly detrimental mutations. There was no significant difference between the effect of new mutations on the wild-type background and the genetically loaded background for four out of five fitness correlates. In these four cases, the maximum level of epistasis compatible with the data was very low. The fifth trait, late productivity, is not likely to be an important component of fitness. This suggests either that specific environmental conditions are required to cause epistasis or that synergistic epistasis is not a general phenomenon. We also suggest a new mechanism by which deleterious mutations may provide an advantage to sexual reproduction under low selection coefficients.

DELETERIOUS mutations are postulated to play an important part in a variety of evolutionary processes. In particular, the distribution of fitnesses in populations at mutation-selection equilibrium is an important component of models of the evolution of sex and recombination (reviews in Kondrashov 1993; Feldman *et al.* 1997; Barton and Charlesworth 1998; Otto and Michalakis 1998), ploidy levels (Kondrashov and Crow 1991), and senescence (Partridge and Barton 1993), as well as of extinction rates in small populations (Lynch and Gabriel 1990; Lynch *et al.* 1995, 1996). These theoretical considerations have led to a number of studies intended to infer the rates and distributions of effects of mutations affecting fitness traits (reviews in Drake *et al.* 1998; Garcia-Dorado *et al.* 1999; Keightley and Eyre-Walker 1999; Lynch *et al.* 1999; Bataillon 2000).

In sexual populations, however, mean fitness at equilibrium depends not only on the rate of mutation, but on the degree of epistasis among mutations (Kimura and Maruyama 1966; Crow 1970; Eshel and Feldman 1970; Kondrashov 1982). This effect is best known for the potential explanation it provides for the maintenance of sexual reproduction: If mutations interact synergistically, such that the proportional effect of a single mutation increases as the number of background mutations increases, then the mean fitness of a sexual population is expected to be greater than that of an asexual population. If the mutation rate is high enough, this effect may provide an advantage to sexual reproduction that can counterbalance the twofold cost of sex or allow the spread of alleles that increase the recombination rate (Felsenstein 1965; Crow 1970; Feldman *et al.* 1980; Kondrashov 1982, 1984, 1985, 1988; Charlesworth 1990; Barton 1995).

It remains unclear, however, whether synergistic epistasis is common enough to make such models generally applicable. Deleterious mutations may also interact antagonistically, such that the proportional reduction in fitness due to a mutation decreases as the number of background mutations increases. In fact, individual pairs of loci that interact in both of these ways almost certainly exist, so the question is really one of the distribution of effects of epistasis across the entire genome. Experiments to detect such a statistical effect of epistasis among mutations have taken two basic forms. One recent approach depends upon the expected distribution of fitnesses among the sexually produced offspring of a pair of parents in haploid organisms (de Visser *et al.* 1996, 1997a; de Visser and Hoekstra 1998; West *et al.* 1998). Although this approach is potentially a powerful way to detect the presence and direction of epistasis in a wide variety of organisms with manageable experimental designs, it provides no clear method for estimating the average magnitude of epistasis between pairs of mutations. In addition, recent experiments of this type (de Visser *et al.* 1996, 1997a; de Visser and Hoekstra 1998) have used variations of the approach for which interpretation of the results can be difficult (West *et al.* 1998). In general, while the results of these experiments may be interpreted as suggesting that epistasis between deleterious mutations is weakly synergistic on average, they should be treated with caution.

Another approach is to determine directly whether or not the logarithm of fitness is an additive function

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of mutation number. This can be achieved by accumulating mutations in such a way that, although the actual number of mutations is not known, treatment groups are expected to be related linearly to mutation number (Mukai 1969) or by generating lines that carry known numbers of mutations (de Visser *et al.* 1997b; Elena and Lenski 1997; Elena 1999). Mukai (1969) found evidence for a nonlinear decline in log fitness over multiple generations of mutation accumulation in *Drosophila melanogaster*. However, uncontrolled factors such as transposable element insertion make this experiment difficult to interpret (Keightley 1996; Keightley and Eyre-Walker 1999), and the estimated degree of epistasis appears to be too high to be consistent with observed levels of inbreeding depression (Charlesworth 1998). More generally, estimates of the average magnitude of epistasis per mutation pair are difficult to extract from designs of this type. This problem can be overcome for experiments making use of known sets of mutations. In four recent experiments, fitness was measured in lines with known numbers of mutations: transposable element insertions in *Escherichia coli* (Elena and Lenski 1997), visible marker mutations in *Aspergillus niger* (de Visser *et al.* 1997b) and *D. melanogaster* (Whitlock and Bourguet 2000), and sequence differences in an RNA virus (Elena 1999). In the case of *D. melanogaster*, a strong statistical effect of synergistic epistasis was driven by one line carrying a full complement of markers, suggesting that some sets of mutations exhibit strong synergistic epistasis, but that the results of such experiments may be sensitive to the specific mutations involved (Whitlock and Bourguet 2000). In the other experiments, there was no net effect of epistasis on average. More detailed analyses in *E. coli* and *A. niger* suggested that most pairs of mutations exhibited either synergistic or antagonistic epistasis, but that these interactions tended to cancel each other out. Although this approach affords a very detailed picture of the magnitude of epistasis and the specific interactions underlying the overall statistical pattern of epistasis, it is limited by being restricted to very small numbers of mutations, as well as to mutations that may not be representative of spontaneous mutations occurring in natural populations. Overall, the picture that emerges from these studies is that if there is net epistasis among deleterious mutations, it is likely to be weak on average.

Here, we present an experiment to test for epistasis among deleterious mutations in the hermaphroditic soil-living nematode *Caenorhabditis elegans*. We subjected worms from two genetic backgrounds—an unmutagenized strain (the unloaded background) and a collection of lines that had been mutagenized in a previous experiment (the loaded background; Davies *et al.* 1999)—to identical mutagenesis procedures with the point mutagen ethyl methanesulfonate (EMS). After inbreeding mutagenized and unmutagenized lines to homozygosity, we measured five fitness correlates and compared changes due to mutagenesis on the loaded background to those on the unloaded background. This approach has several advantages. First, the mutagenesis procedure induces large numbers of mutations; thus, even weak epistasis should be detectable. Second, the mutations induced are primarily G/C → A/T transitions and should be located randomly throughout the genome; such mutations are expected to be similar in their fitness consequences to a random sample of spontaneous point mutations (Davies *et al.* 1999). Finally, there is an *a priori* expectation that the mutagenesis procedure should generate at least 45 mutations per line that are deleterious under natural conditions (Anderson 1995; Davies *et al.* 1999); using this quantitative expectation, we can obtain an estimate of the average epistasis per pair of deleterious mutations, although we are unable to make inferences about the distribution of epistatic effects.

MATERIALS AND METHODS

Background lines and culture conditions: In a previous experiment (Davies *et al.* 1999), individuals of the N2 strain of *C. elegans* were exposed to a 50 mm dose of EMS for 4 hr at 20°. This procedure generates ~220 G/C → A/T transitions, approximately 45 [95% confidence interval (CI) 29–61] of which are expected to cause deleterious amino-acid changes (Davies *et al.* 1999). Sixty of these individuals and 40 unmutagenized individuals were then chosen at random to generate experimental and control lineages, which were then inbred under minimized selection for 10 generations to ensure that the mutations were in the homozygous state. For the present experiment, one lineage was chosen arbitrarily from among the 40 control lineages to act as an unloaded background, while the 24 lineages with the highest calculated value of r (the intrinsic rate of population increase) among the 56 surviving experimental lineages were chosen to act as a loaded background.

These lines, which had been frozen at −85°, were thawed and maintained throughout the experiment at 20°, on 3.5-cm agar plates seeded with *E. coli* strain OP50, using standard techniques (Sulston and Hodgkin 1988). Prior to mutagenesis, each line was maintained for four generations by randomly choosing four individuals at the L3 larval stage and transferring them to a new plate.

Mutagenesis: On the fifth generation after thawing, the unloaded background line was split into 24 sublines, and all lines (24 loaded-background lines and 24 unloaded-background sublines) were synchronized by immersing gravid adults in an alkaline-hypochlorite solution (Sulston and Hodgkin 1988) and allocating their eggs to fresh agar plates to hatch. When these worms reached the L4 larval stage, a subset of the worms from each line was mutagenized. The basic mutagenesis procedure followed the protocol set out in Anderson (1995) and Davies *et al.* (1999): Worms from each line were suspended in a 50 mm solution of EMS in M9 buffer for 4 hr and then washed three times with M9 and transferred to fresh plates. To minimize timing and block effects associated with mutagenizing 48 lines, mutagenesis was performed on six blocks, each consisting of four loaded and four unloaded background lines, chosen at random and interleaved. The time between the start of the first and last mutagenesis within each block was 14 min; the total time between the start of the very first and the very last mutagenesis was 2 hr, 59

min. Each mutagenized line received its first M9 wash within 1 min of 4 hr after the addition of EMS. Unmutagenized and mutagenized individuals from each background line were then transferred to separate plates, giving 24 plates in each of four background-by-treatment combinations: unloaded unmutagenized (00); loaded unmutagenized (10); unloaded mutagenized (01); and loaded mutagenized (11).

Propagation of lines and inbreeding: Three days after the mutagenesis, one L3 larva was chosen at random from among the offspring on each plate, to serve as the progenitor of a new line. The lines were then propagated by transferring one larval hermaphrodite to a fresh plate every generation. This design minimizes selection and generates homozygous offspring by sending each line through a bottleneck of one self-fertilizing individual every generation. Extra plates (one per unmutagenized line and two per mutagenized line) were set up each generation; offspring were chosen at random from these plates in the event that a worm failed to reproduce. If no backup plate had produced, plates from the previous generation were used, to a maximum of three previous generations. Transfers were continued until each line had gone through at least 10 transfers; because some mutagenized lines produced very slowly, the unmutagenized (00 and 10) lines were taken through 13 transfers while the mutagenized (01 and 11) lines were taken through 10 transfers.

Inference of rate of loss of mutations due to selection: Mortality during this inbreeding phase presents an opportunity for selection to act. To estimate the number of mutations lost to selection, we performed computer simulations of an experiment identical in design to that presented here to determine what patterns of selection are consistent with observed mortality during inbreeding (Davies *et al.* 1999). EMS mutagenesis was assumed to induce a Poisson mean of n mildly deleterious, and 1 near-lethal ($s = 0.9$), mutations per haploid, the latter as expected for 50 mM EMS (Rosenbluth *et al.* 1983). The effects of minor mutations were assumed to be a constant s_m. Each mutation was assigned to a position on the genetic map by choosing a random physical position on a random chromosome and then calculating the map position using interpolation functions based on "Marey maps" (Barnes *et al.* 1995). One individual per line per generation was assumed to reproduce by selfing; the recombinant frequency per chromosome per generation was exactly 0.5, with crossovers occurring at random locations. An individual's probability of surviving to reproduce was calculated as $v_c \times \Pi_i(1 - hs_i) \times \Pi_j(1 - s_j)$, where v_c is the mean observed viability for the unloaded-unmutagenized (00) lines (0.94), s is the mutation effect, h is the dominance coefficient, and i and j are indices of heterozygous and homozygous mutations, respectively. Highly deleterious mutations ($s > 0.5$) were assumed to be completely recessive ($h = 0$); for all other mutations, $h = 0.2$. Previous examinations of such simulations have shown that the results are insensitive to changes in h or differences in the distribution of s_m (Davies *et al.* 1999). If an individual died, one of two backup individuals was used; if neither backup survived, an offspring from the previous generation was used, and so on up to three previous generations. One thousand simulations were run for each of several values of n and s_m. For each set of parameter values, the mean proportion of plates surviving (v) and the mean number of mutations lost to selection (m_l) per line were calculated. If the mean survivorship v for a given set of parameter values fell within the observed 95% confidence interval for a treatment group in the experiment, those parameter values were considered to be consistent with the observed results; the values of m_l associated with these parameter values give an estimate of the range of numbers of mutations lost to selection.

Trait assays: After the inbreeding phase, each line was split into three replicate sublines; lines within each of the three sets of replicates were randomized and taken through three generations of larval transfers to remove effects of common environment. Lines were then synchronized by placing gravid adults onto plates for ~3 hr and allowing them to lay eggs. These eggs were allowed to develop to the L4 larval stage; one larva was then chosen at random per replicate per line and moved to a new plate for measurements of daily productivity and longevity. Every day for 5 days, each worm was moved to a fresh plate; the eggs on each plate were allowed to hatch and the progeny counted, giving daily productivity over 5 days. The day of death of the assayed worm was also noted. If a worm was accidentally killed, an additional replicate of the appropriate lines was added to the next assay. The entire assay was repeated on the same sublines two more times, with the exception that in the second assay synchronization was carried out by immersing gravid adults in alkaline-hypochlorite solution (Sulston and Hodgkin 1988) to remove bacterial contamination. There were a total of 3 replicates × 3 assays = 9 measurements/line.

Fitness correlates: Trait measurements were used to calculate five fitness correlates for each worm: total productivity; early productivity (days 1–2 of the reproductive period); late productivity (days 3–5 of the reproductive period); longevity; and relative fitness (w). Relative fitness is a measure of fitness appropriate for age-structured populations at equilibrium; it is defined as

$$w = \sum_x e^{-r_c x} l_x m_x,$$

where r_c is the intrinsic rate of increase of the control (00) population, calculated by solving the equation $\Sigma_x e^{-r_c x} l_x m_x = 1$. l_x and m_x are the proportion of individuals surviving to day x and the expected number of progeny produced by surviving individuals on day x, respectively (Charlesworth 1994). For the calculation of r_c, l_x and m_x were calculated across all individuals in the control (00) group, while for the calculation of w, they were calculated for each individual.

Analysis: The effects of epistasis among mutations were estimated in three ways: by comparing the changes in mean and genetic variance of mutagenized individuals on the two different backgrounds; by estimating mutation rates and effects in the various background-by-treatment combinations using maximum likelihood; and by testing for an interaction between background and treatment in an analysis of variance on log-transformed trait values.

Changes in mean and variance: Least-squares means of untransformed trait values for each background-by-treatment combination were calculated using the MIXED procedure of SAS 6.1.2 (SAS Institute 1990). Factors included in the model were background-by-treatment combination (00, 01, 10, and 11), replicate, assay, line (nested within background-by-treatment), replicate*line, and replicate*assay. Line and replicate*line were treated as random effects; all other effects were treated as fixed.

Genetic variances in log-transformed trait values (V'_G) were calculated separately for each background-by-treatment combination. To remove scaling effects (*i.e.*, a reduction in variance as the trait mean approaches the absorbing state of zero), trait values X were transformed by $\ln(X + c)$, where c is 0 for longevity, 0.01 for w, and 1 for all other traits. The constant c was required to allow the inclusion of zeroes in the analysis; the standard value of c is 1 (Zar 1984), but a smaller value (0.01) was used in the case of w because the mean value of w is less than 1. A constant was not required for longevity data because there were no zero values. V'_G was calculated using the REML algorithm of the VARCOMP procedure of SAS 6.1.2 (SAS Institute 1990). The statistical model was the same as

that for the calculation of least-squares means described above, except that since a separate analysis was done for each background-by-treatment combination, line was not nested.

To compare the two mutagenized groups (01 and 11) to their backgrounds (00 and 10), the proportional change in mean of untransformed trait values, $\Delta X = (\bar{X}_{i0} - \bar{X}_{i1})/\bar{X}_{i0}$, and the change in variance of transformed trait values, $\Delta V'_G = V'_{G,i1} - V'_{G,i0}$, were calculated, where $i = 0$ for the unloaded background and $i = 1$ for the loaded background. Differences in these quantities between the two backgrounds may be construed as evidence of epistasis; significance of such differences was tested by bootstrapping the entire dataset 1000 times, resampling by background line (*i.e.*, always drawing both mutagenized and unmutagenized lines from a given background line) to maintain the correlation structure within a background.

Maximum likelihood estimation of mutational parameters: Maximum likelihood (ML) can be used to estimate the numbers of mutations induced by the EMS treatment and their average effects (Keightley and Ohnishi 1998). Epistasis may be detected as a difference in the number or magnitude of effects on the loaded compared to the unloaded background. For a given trait, the data on line means from all four treatments were analyzed simultaneously using a multiplicative model. The line means for unloaded-unmutagenized (00) lines were assumed to be normally distributed with variance σ^2_e. The distribution of the line means in the unloaded-unmutagenized treatment shows no significant deviation from normality in any trait when tested with a Shapiro/Wilk test (SAS Institute 1990). The loaded-unmutagenized (10) lines were assumed to have the same environmental variance and to contain an average of U_{10} mutations of effect s_{10}, with the number of mutations given by a Poisson deviate. The unloaded-mutagenized (01) lines were assumed to contain U_{01} mutations (again Poisson distributed) with effects s_{01}. The loaded-mutagenized (11) lines contained U_{11} mutations in addition to their load of U_{10} mutations, and these had separate effects s_{11} and s_{10}. The covariance structure between the loaded-unmutagenized lines and loaded-mutagenized lines was incorporated by assuming a "two-generation" experiment as described by Keightley and Bataillon (2000). To test for epistasis, the likelihood of the full model described above in which all mutation numbers (U_{10}, U_{01}, and U_{11}) and all effects (s_{10}, s_{01}, and s_{11}) were estimated, was evaluated. This was compared to the likelihood of a restricted model in which the mutation numbers and effects of mutagenesis on the loaded and unloaded backgrounds were the same; *i.e.*, the parameters estimated were U_{10}, $U_{01} = U_{11}$, s_{10}, $s_{01} = s_{11}$. The models differ by two parameters, so the test for epistasis was based on a chi-square distribution with 2 d.f. This analysis was run on all traits except late productivity, because the analysis assumes unidirectional effects, while mutational effects on late productivity were clearly bidirectional.

Analysis of variance: Epistasis among mutations should also appear as an interaction between background and treatment on the log scale. Trait values were transformed as described above; mixed-model ANOVAs fitting the effects of background, treatment, background line, treatment line (nested within background line), assay, replicate, and the background*treatment interaction were run using the MIXED procedure of SAS 6.1.2 (SAS Institute 1990). Treatment line was treated as a random effect; all other effects were treated as fixed. Estimates of expected additive effects of background and treatment mutagenesis (the sum of the reduction in transformed trait values in the two single-mutagenesis groups 10 and 01) and deviation from additivity (the interaction effect: any deviation from additivity in the double-mutagenesis group 11) were calculated as contrasts within the MIXED procedure.

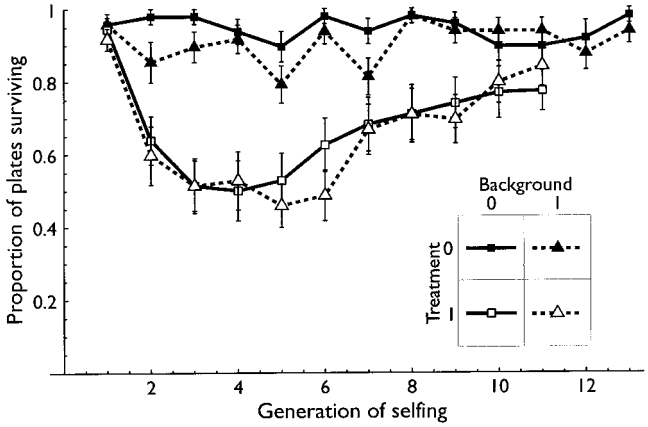

Figure 1.—Mean survivorship during 13 generations of inbreeding. Unloaded background: solid lines, boxes. Loaded background: dashed lines, triangles. Unmutagenized treatment: solid points. Mutagenized treatment: open points.

RESULTS

Selection during inbreeding: Both mutagenized groups (01 and 11) showed increased mortality during the selfing phase (Figure 1). Mortality increased steeply at first, then decreased, a pattern that suggests purging of mutations as they are exposed in the homozygous state. Total mortality (proportion of plates failing to produce) over all generations was very similar between the two mutagenized groups (01 and 11, Table 1). Assuming that EMS mutagenesis produced a Poisson mean of 45 mildly deleterious and one near-lethal mutation per line (Rosenbluth *et al.* 1983; Davies *et al.* 1999), this pattern of mortality is consistent with the loss of $\leq$4.1 mildly deleterious mutations per line in our computer simulations (Table 1). If it is assumed that EMS mutagenesis induced less than or greater than 45 mutations per line, the proportion of mutation lost remains on the order of 8–9% (Table 1). There is no evidence that selection removed more mutations in double-mutagenized lines (group 11) than in single-mutagenized lines (group 01), although we cannot detect selection on the egg-to-hatching phase of the life cycle.

Changes in mean and variance: Mutagenesis with EMS significantly decreased the mean of most traits on either genetic background (Figure 2, Table 2), with several notable exceptions. Mutagenesis did not significantly decrease mean late productivity on the unloaded background; there was a significant effect on the loaded background, but this may be due to one loaded-unmutagenized line that had very high late productivity (Figure 2C, Table 2C). Mutagenesis also has no significant effect on mean longevity on either background (Figure 2E, Table 2E). Genetic variance of $\ln(X + c)$-transformed trait values (V'_G) increased significantly with mutagenesis on both backgrounds for all traits, with the exception of longevity on the loaded background, in which V'_G did not change significantly (Table 3).

TABLE 1

Rates of mortality in mutagenized lines during inbreeding phase and simulation-based inferences of properties and numbers of mutations lost to selection, assuming EMS mutagenesis induced 20, 45, or 80 mutations

U	Background	Total observed mortality (%)	Selection coefficient (%)[a]	Proportion of mutations lost (%)[a]	No. of minor mutations lost[a,b]
20	Unloaded	37.1 (28.5–45.6)	1.34 (0.96–1.84)	8.2 (5.8–10.6)	0.64 (0.16–1.11)
	Loaded	37.8 (30.2–25.4)	1.38 (1.04–2.99)	8.4 (6.3–10.5)	0.68 (0.26–1.10)
45	Unloaded	37.1 (28.5–45.6)	0.22 (0.15–0.30)	8.8 (6.4–11.2)	3.0 (1.9–4.1)
	Loaded	37.8 (30.2–45.4)	0.23 (0.17–0.29)	9.0 (6.9–11.2)	3.1 (2.1–4.0)
80	Unloaded	37.1 (28.5–45.6)	0.066 (0.050–0.089)	9.0 (6.5–11.4)	6.2 (4.2–8.1)
	Loaded	37.8 (30.2–45.4)	0.068 (0.052–0.088)	9.2 (7.0–11.3)	6.3 (4.6–8.1)

[a] Obtained by comparison with simulation results.
[b] Assuming a Poisson mean of U EMS-induced mutations of minor effect ($s < 0.5$) and one of major effect ($s > 0.5$) per haploid genome.

We compared the proportional decrease in trait mean as a result of mutagenesis on the loaded background to that on the unloaded background and tested for a significant difference by bootstrapping by line (Table 2). The decrease due to mutagenesis tends to be greater on the loaded background than the unloaded background for all traits except longevity, although the difference is only significant for late productivity.

Comparisons of the change in V'_G suggest that there is no difference between the two backgrounds in most cases (Table 3). Although mutagenesis increases the point estimate of V'_G less on the loaded background than the unloaded background for most traits, this pattern is nonsignificant for all traits except longevity, which is marginally significant; the pattern is reversed (but still nonsignificant) in the case of w.

Estimates of mutational parameters: A full model, in which EMS-induced mutation rates (U_{01} and U_{11}) and effects (s_{01} and s_{11}) were allowed to differ on the two genetic backgrounds, did not fit significantly better than a reduced model, in which mutation rates and effects on the two backgrounds were constrained to be equal, for any trait (Table 4). The changes in log likelihood are so small as to imply that models in which only one parameter was allowed to vary across backgrounds (*i.e.*, $U_{01} = U_{11}$ or $s_{01} = s_{11}$) would also not fit significantly better than the reduced model. Thus, the maximum likelihood estimates of mutational parameters suggest that there is no significant effect of epistasis. The estimates for total productivity are consistent with previous estimates (Keightley and Caballero 1997; Davies *et al.* 1999): The estimated number of new mutations in the mutagenized treatments (01 and 11) is about two to three per individual, with effects of ~22–24% (Table 4).

It is notable that the estimated mutation rate is about twice as large, while the estimated effect is about half as large, on the loaded background than on the unloaded background (*i.e.*, $U_{11} \approx 2U_{01}$ and $s_{11} \approx s_{01}/2$) for both early productivity and relative fitness under the full model (Table 4). Although this difference is not significant (*i.e.*, the full model does not fit significantly better than the reduced model), it does suggest the possibility that mutations that do not contribute to measured variation on the unloaded background are more likely to contribute to variation on the loaded background. We have suggested that a large proportion of deleterious mutations have effects that are too small to be detected under laboratory conditions (Davies *et al.* 1999). If synergistic epistasis renders some of these mutations measurable on the loaded background, the effect should be to increase the estimated number of mutations while decreasing their average effects, because a larger number of mutations with very small effects would be contributing to variation. Thus, the parameter estimates resulting from the full model are consistent with a specific form of synergistic epistasis, although this effect is not significant.

Analysis of variance: We used analysis of variance to test for a significant interaction between background and treatment on $\ln(X + c)$-transformed values of each trait. Table 5 shows least-squares mean values for all groups (Table 5A), as well as estimates of the additive effects of the background and treatment mutagen doses (A, the total expected reduction under a log-linear fitness function) and the background*treatment interaction effect (B, the total deviation from linearity) (Table 5B). A positive interaction effect corresponds to a larger difference on the loaded background and is evidence for synergistic epistasis. Although the estimate of this effect is positive for all traits except longevity, it is only marginally significant in the case of late productivity.

The case of late productivity is notable, because the linear effect A is nonsignificant while the interaction effect B is marginally significant, and the estimate of the interaction effect is more than twice as large as that of the linear effect (Table 5). This suggests strong

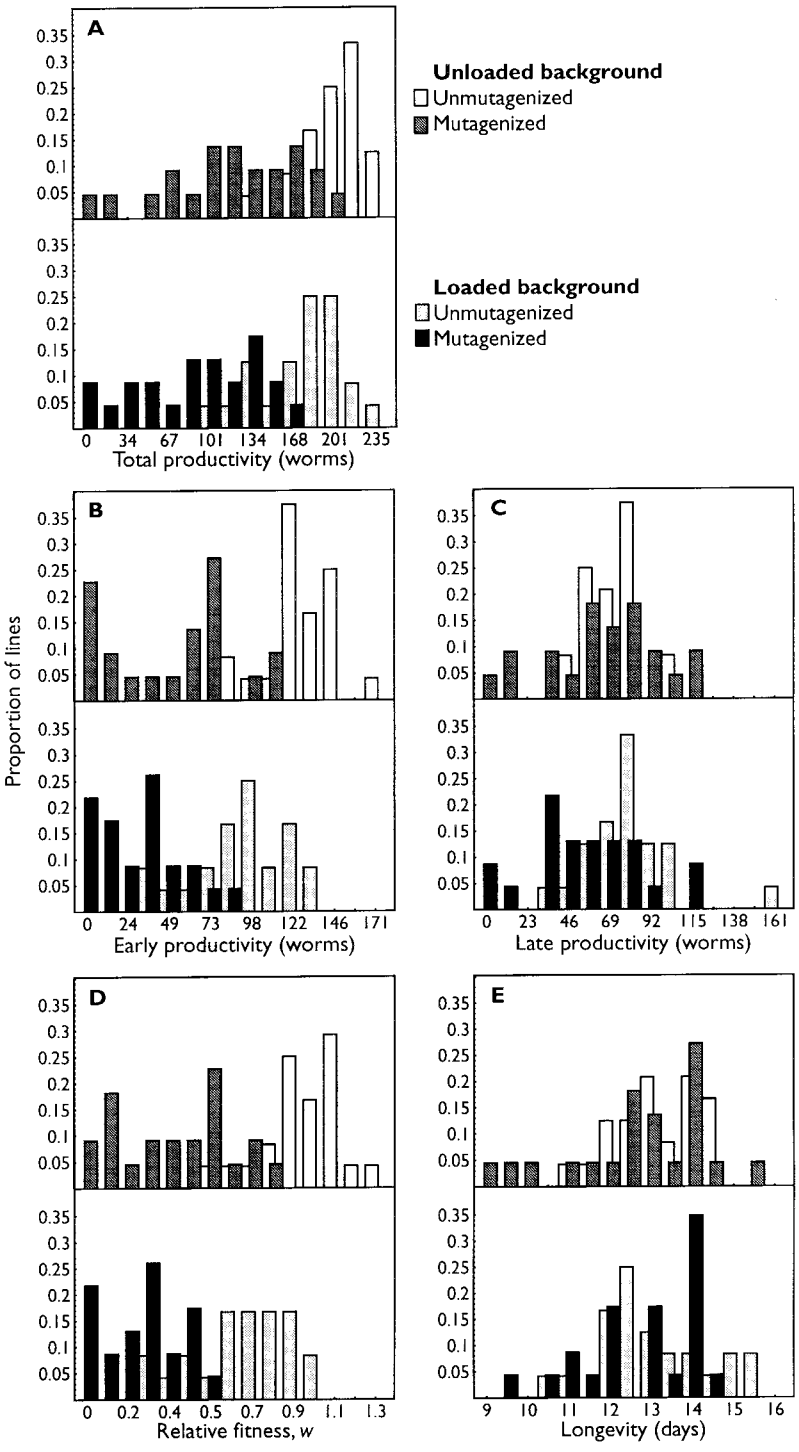

FIGURE 2.—Distribution of line means. (A–E) Top shows unloaded-background lines; bottom shows loaded-background lines. Solid bars correspond to mutagenized treatment. (A) Total productivity; (B) early productivity; (C) late productivity; (D) relative fitness; (E) longevity.

synergistic epistasis: The expected total effect of two single mutagen doses is no decrease in late productivity, while a double dose causes a substantial decrease. However, late productivity is not particularly reliable as a fitness correlate. Nonetheless, this result points out a potential pattern in timing of reproduction as a response to mutagenesis. Mutations may act to both delay reproduction and decrease total productivity, causing the peak in reproduction to occur later, and be smaller, in mutagenized individuals. Since late productivity is lower than early productivity in control worms (Table 2), this causes a relatively small net change in late productivity as a result of a single mutagenesis. However, with the additional delay and decrease in productivity resulting from a second mutagenesis, late productivity drops substantially, leading to the large interaction term. Thus, a pattern that is associated with largely nonepistatic effects in traits that are closely related to fitness (early and total productivity) can cause epistasis in other traits (late productivity).

TABLE 2

Least-squares trait means (SEs) by background and treatment, and proportional decrease in trait mean within backgrounds, $\Delta\bar{X} = (\bar{X}_{control} - \bar{X}_{treated})/\bar{X}_{control}$

Trait (units)	Treatment	Background: Unloaded	Background: Loaded	P^a
A. Total productivity (worms)	Unmutagenized	210.5 (8.12)	185.9 (8.12)	
	Mutagenized	125.8 (8.49)	97.5 (8.38)	
	$\Delta\bar{X}$	0.40 (0.042)***	0.48 (0.037)***	0.10
B. Early productivity (worms)	Unmutagenized	133.1 (5.55)	100.0 (5.55)	
	Mutagenized	57.0 (5.81)	37.3 (5.74)	
	$\Delta\bar{X}$	0.57 (0.043)***	0.64 (0.038)***	0.21
C. Late productivity (worms)	Unmutagenized	77.5 (5.34)	85.8 (5.34)	
	Mutagenized	68.8 (5.58)	60.5 (5.52)	
	$\Delta\bar{X}$	0.11 (0.074)	0.30 (0.056)***	0.03
D. Relative fitness, w	Unmutagenized	1.00 (0.040)	0.749 (0.040)	
	Mutagenized	0.435 (0.042)	0.285 (0.042)	
	$\Delta\bar{X}$	0.57 (0.041)***	0.63 (0.038)***	0.28
E. Longevity (days)	Unmutagenized	13.3 (0.285)	13.2 (0.287)	
	Mutagenized	12.7 (0.304)	12.9 (0.298)	
	$\Delta\bar{X}$	0.047 (0.025)	0.026 (0.022)	0.57

[a] P values represent the difference in response on the two backgrounds and are calculated as $P = 2a$, where a is the proportion of bootstrap replicates out of 1000 in which the proportional decrease in mean was greater or less on the unloaded background than on the loaded background, whichever value is <0.5. This accounts for the two-tailed nature of the test.

*** Significant effect of mutagenesis within a background, $P < 0.0006$.

Effects of development rate: It is possible that variation in development rate among loaded background lines could bias our results: If worms of different lines were at slightly different developmental stages when they were mutagenized, EMS mutagenesis may have had more effect on some lines than on others. We minimized

TABLE 3

V'_G, genetic variance in $\ln(X + c)$-transformed traits, by background and treament, and change in genetic variance of $\ln(X + c)$-transformed traits within backgrounds, $\Delta V'_G = V'_{G,treated} - V'_{G,control}$

Trait (units)	Treatment	Background: Unloaded	Background: Loaded	P^a
A. Total productivity [ln(worms)^2]	Unmutagenized	0.0 (—)	0.0082 (0.021)	
	Mutagenized	0.98 (0.38)	0.94 (0.40)	
	$\Delta V'_G$	1.00 (0.37)***	0.93 (0.39)***	0.88
B. Early productivity [ln(worms)^2]	Unmutagenized	0.043 (0.056)	0.099 (0.067)	
	Mutagenized	1.3 (0.52)	1.3 (0.49)	
	$\Delta V'_G$	1.34 (0.32)***	1.21 (0.40)***	0.87
C. Late productivity [ln(worms)^2]	Unmutagenized	0 (—)	0.11 (0.059)	
	Mutagenized	0.89 (0.34)	0.50 (0.25)	
	$\Delta V'_G$	0.89 (0.056)***	0.39 (0.077)***	0.31
D. Relative fitness, $\ln(w)^2$	Unmutagenized	0.016 (0.043)	0.062 (0.052)	
	Mutagenized	1.4 (0.56)	1.9 (0.72)	
	$\Delta V'_G$	1.41 (0.45)***	1.81 (0.68)***	0.50
E. Longevity [ln(days)^2]	Unmutagenized	0.0011 (0.0025)	0.0020 (0.0031)	
	Mutagenized	0.018 (0.0090)	0.0036 (0.0045)	
	$\Delta V'_G$	0.017 (0.022)***	0.0016 (0.026)	0.07

[a] P values represent the difference in response on the two backgrounds and are calculated as $P = 2a$, where a is the proportion of bootstrap replicates out of 1000 in which the change in V'_G was greater or less on the unloaded background than on the loaded background, whichever value is <0.5. This accounts for the two-tailed nature of the test.

*** Significant effect of mutagenesis within a background, $P < 0.0006$.

TABLE 4

ML estimates of mutational parameters and fit of full model compared to reduced model

Trait	Model	U_{10}	U_{01}	U_{11}	s_{10}	s_{01}	s_{11}	$\Delta\text{Log } L^a$	P^b
Total productivity	Full[c]	0.42	2.0	2.9	0.22	0.24	0.22	0	
	Reduced[d]	0.90	2.3		0.13	0.23		−0.3	0.85
Early productivity	Full[c]	2.6	3.1	5.7	0.11	0.28	0.16	0	
	Reduced[d]	2.1	3.9		0.14	0.22		−0.5	0.78
Relative fitness, w	Full[c]	3.3	4.3	9.3	0.092	0.20	0.10	0	
	Reduced[d]	3.0	5.6		0.10	0.15		−0.3	0.86
Longevity	Full[c]	0.19	0.17	0.00	0.071	0.26	0.17	0	
	Reduced[d]	0.050	0.16		0.093	0.21		−1.2	0.55

[a] Relative to full model.
[b] Based on a chi-square distribution with 2 d.f.
[c] All parameters free.
[d] $U_{11} = U_{01}$; $s_{11} = s_{01}$.

this effect experimentally by selecting background lines that had fitnesses similar to that of wild-type lines and would therefore be expected to have similar rates of development; we also chose worms at the same larval stage (L4) for mutagenesis. However, the L4 larval stage of wild-type worms lasts ~12.25 hr at 20° (Lewis and Fleming 1995); if the effectiveness of EMS varies with age within the L4 stage, there is still some potential for bias. To test this, we performed a linear regression of least-squares line means of each trait against the time at which the mutagenesis was originally performed. Since the total time span between the first and last mutagenesis was ~3 hr, and worms were the same age at the beginning of that time, differences in susceptibility within the L4 stage should appear as a significant effect of timing on trait values. This analysis was performed on both raw means and means relative to the background line. For no trait was there a significant relationship between trait values and time (smallest $P = 0.29$, largest $r^2 = 0.056$) for mutagenized worms on either background (Figure 3), suggesting that developmental stage at mutagenesis was unlikely to be an important source of variation in trait values.

DISCUSSION

Summary of results and relation to theoretical parameters: Overall, if there is a net effect of epistasis among deleterious mutations, it is too weak to be detected in this experiment, although the trend is toward synergistic epistasis. Comparisons of the change in mean and variance as a result of mutagenesis show no significant difference on the loaded *vs.* the unloaded background for any trait except late productivity, which is not expected

TABLE 5

Summary of least-squares mean and effect estimates from ANOVA on $\ln(X + c)$-transformed trait values

Group/effect	Total productivity	Early productivity	Late productivity	w	Longevity
		A. Least-squares means			
Unmutagenized background					
Control (00)	5.24	4.54	3.96	−0.28	2.56
Treated (01)	4.22	2.95	3.56	−1.88	2.50
Mutagenized background					
Control (10)	5.10	4.22	4.16	−0.60	2.55
Treated (11)	3.85	2.49	3.33	−2.42	2.51
		B. Effect estimates (SE)			
Additive effect, A^a	1.157***	1.909***	0.202	1.930***	0.078
	(0.248)	(0.292)	(0.252)	(0.315)	(0.049)
Interaction effect, B^b	0.230	0.141	0.418*	0.216	−0.025
	(0.225)	(0.266)	(0.226)	(0.287)	(0.042)

* $P < 0.06$, *** $P < 0.0001$.
[a] A represents the expected reduction in log-transformed trait values of double-mutagenized (11) lines if the mutations in the two mutagenesis treatments do not interact.
[b] Positive interaction effects indicate a greater reduction on the loaded background than on the unloaded background, *i.e.*, synergistic epistasis.

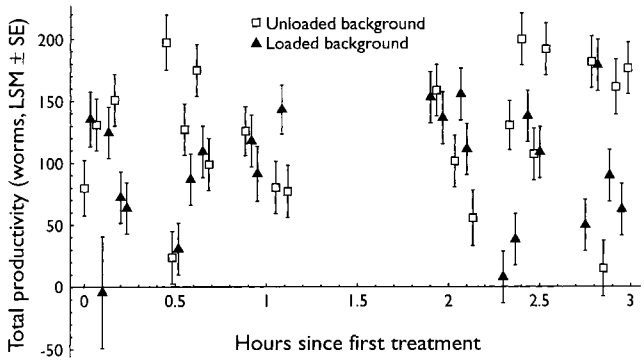

FIGURE 3.—Effect of timing on effects of mutagenesis on total productivity. Open squares represent the unloaded background ($F_{1,20} = 0.45$, $P = 0.46$, $r^2 = 0.026$); solid triangles represent the loaded background ($F_{1,21} = 0.40$, $P = 0.53$, $r^2 = 0.019$). This pattern is representative of the effects on all traits, absolute and relative.

to be closely related to fitness (Tables 2 and 3). Similarly, only late productivity showed a marginally significant background*treatment interaction in an analysis of variance on $\ln(X + c)$-transformed data (Table 5). Finally, a model in which mutation rates and effects were allowed to vary across the two backgrounds did not fit significantly better than one in which they were constrained to be equal, for any trait analyzed using maximum likelihood (Table 4).

Although none of these results showed significant effects of epistasis, we may still calculate the range of epistatic effects that is consistent with the data. We do so here by determining the 95% confidence intervals around the effect estimates from the ANOVA on $\ln(X + c)$-transformed data (Table 5B). The effects of interest are the additive effect A, which is the reduction in fitness expected in the double-mutagenized (11) group if there were no interactions between the two sets of mutations, and the background*treatment interaction effect B, which is the net effect of epistasis on the double-mutagenized (11) group. Taking total productivity as an example, our estimate of the additive effect is $A = 1.157$ (95% CI 0.671–1.643); our estimate of the net effect of epistasis is $B = 0.230$ (95% CI −0.211–0.671) (Table 5B). We can also calculate the effect of epistasis scaled by the additive effect, B/A. Confidence intervals on this quantity can be estimated by bootstrapping with the resampling scheme described in MATERIALS AND METHODS. Using this approach, our estimate of the effect of epistasis relative to the additive effect is $B/A = 0.199$ (95% CI −0.253–1.07).

Estimates of A and B can be used to calculate the average additive effect per mutation (α) and the average effect of epistasis per pair of mutations (β), which are important parameters in some models of sex and recombination (CHARLESWORTH 1990). We define the number of mutations in the loaded background as U_{10} and the number in the mutagenized treatment as U_{01}; the mean linear effect per mutation is then $\alpha = A/(U_{10} + U_{01})$. The number of pairs of mutations contributing to the interaction between background and treatment is $U_{10} \times U_{01}$, so the mean epistasis per pair of mutations is $\beta = B/U_{10}U_{01}$. The degree of epistasis is the epistatic coefficient scaled by the additive coefficient, β/α. These values apply to homozygous mutations; following CHARLESWORTH (1990), heterozygous effects can be estimated by scaling by the dominance coefficient h, such that $\alpha_{het} = \alpha h$, $\beta_{het} = \beta h^2$, and $(\beta/\alpha)_{het} = (\beta/\alpha)h$.

The predicted number of deleterious amino-acid-altering mutations induced by each mutagen treatment is 45 (ANDERSON 1995; DAVIES *et al.* 1999). Setting $U_{10} = U_{01} = 45$ and using the effect estimates and confidence intervals from above give $\alpha = 0.013$ (95% CI 0.007–0.018), $\beta = 1.1 \times 10^{-4}$ (95% CI -1.0×10^{-4} -3.3×10^{-4}), and degree of epistasis $\beta/\alpha = 0.0088$ (95% CI −0.011–0.048) (Table 6). Relaxing the assumption that each mutagen dose produced 45 deleterious mutations can cause estimates of α, β, and β/α to vary across several orders of magnitude, but if this number varies from 45 by a factor of ~2 in either direction (between 20 and 80), the estimate of β/α remains on the order of 10^{-2} or below (Table 6). The maximum degree of epistasis consistent with the data under this range of mutation numbers is $\beta/\alpha = 0.11$ (the upper confidence limit for $U_{10} = U_{01} = 20$, Table 6).

The above calculation depends upon a large number of assumptions that are almost certainly violated. Most important of these assumptions are, first, that the only epistatic interactions are those between mutations induced by separate mutagen doses. The linear effect A is calculated as the sum of the difference in trait values between the two singly mutagenized groups (10 and 01) and the unloaded-unmutagenized group (00). In fact, this sum also includes any epistatic interactions (1) among mutations already present in the unloaded background line (*i.e.*, within group 00); (2) among mutations induced by each single mutagenesis (*i.e.*, within group 10 or 01); and (3) between these sets of mutations (*i.e.*, between groups 00 and 10/01). If this hidden epistasis is synergistic, then the calculated value of A will be larger than the actual linear effect, so α will be overestimated, and β/α underestimated. Note that this does not bias our estimate of β. A second assumption is that mutations have equal effects; in fact, we have suggested that EMS-induced mutations in *C. elegans* have a bimodal distribution of effects, such that the effects of ≥97% of deleterious mutations are unmeasurably small (DAVIES *et al.* 1999). If the measured deviation from linearity is caused primarily by interactions among those relatively few mutations that are measurable, the effective number of mutations U may be overestimated, leading to underestimates of α, β, and β/α.

It is also possible that epistasis among mutations appears only in particular environmental circumstances, such as strong intraspecific competition (KING 1967;

TABLE 6

Estimates of linear (α) and epistatic (β) parameters for total productivity and deleterious mutation rates required to overcome a twofold cost of sex

Nos. of mutations induced					Mutation rate providing advantage to sex	
U_{10}	U_{01}	$(\alpha/h)^a$ (95% CI)	$[(\beta/h^2) \times 10^3]^a$ (95% CI)	$[(\beta/\alpha)/h]^a$ (95% CI)	$U_0^{*b,c}$	$U_i^{*c,d}$
5	5	0.116 (0.06–0.16)	9.20 (−8–30)	0.079 (−0.10–0.42)	2.90	1.08
10	10	0.058 (0.03–0.08)	2.30 (−2–7)	0.040 (−0.05–0.21)	2.86	0.96
20	20	0.029 (0.02–0.04)	0.575 (−0.5–2)	0.020 (−0.02–0.11)	2.84	0.87
30	45	0.015 (0.01–0.02)	0.170 (−0.2–0.5)	0.011 (−0.01–0.05)	2.79	0.82
45	45	0.013 (0.007–0.02)	0.114 (−0.1–0.3)	0.0088 (−0.01–0.05)	2.83	0.81
80	80	0.0072 (0.004–0.01)	0.036 (−0.03–0.1)	0.0049 (−0.01–0.02)	2.81	0.78

[a] Heterozygous coefficients are scaled by dominance coefficient h.
[b] Assumes clonal population begins with same distribution as sexual population or with zero mutations.
[c] Calculations based on equations from Charlesworth (1990), assuming $h = 0.25$ and free recombination in the sexual population.
[d] Assumes clonal population begins with $i = \bar{n} - 2\sigma$ mutations (2 SD below the mean of the sexual population).

Kondrashov 1988; Peck and Waxman 2000). Although we have not examined the effects of varied environment or competition here, these possibilities could certainly be tested in the future. More generally, if specific environmental conditions are required to create epistasis, mutational models for sex lose some of their generality, although they may gain some power to explain the well-known ecological and geographical correlates of sexual reproduction (Bell 1982).

C. elegans is a primarily selfing hermaphrodite and produces obligately outcrossing male offspring at a frequency of ~0.1% under laboratory conditions (Hodgkin *et al.* 1979). As such, it does not pay the full twofold cost of sex (Charlesworth 1980; Lloyd 1980) and may not seem like the ideal model system for testing the assumptions of models of the maintenance of sex. However, the nature of epistasis among mutations seems much less likely to vary from species to species than traits such as mutation rate. Indeed, it has been suggested that at least the sign of epistasis should be partly determined by the nature of selection on metabolic pathways (Szathmàry 1993). Perhaps more convincingly, studies of bacteria (Elena and Lenski 1997), fungi (de Visser *et al.* 1997b), viruses (Elena 1999), and now nematodes all suggest that the net effect of epistasis on fitness is weak at best, although recent estimates of high mutation rates in hominids can be interpreted as implying the existence of synergistic epistasis (Eyre-Walker and Keightley 1999).

Comparison with previous work: Charlesworth (1990) defines log fitness as $\ln(w) = -(\alpha n + \beta n^2/2)$, where n is the number of mutations. Previous estimates of β (de Visser *et al.* 1997b; Elena and Lenski 1997; Elena 1999) are measured as the quadratic coefficient in a regression of fitness against mutation number, assuming $\ln(w) = -(\alpha n + \beta n^2)$; thus, for comparison with Charlesworth's (1990) model, β from such analyses should be multiplied by 2. Our estimates of β are measured per pair of mutations, giving the underlying fitness model

$$\ln(w) = -\left(\alpha n + \beta \frac{n(n-1)}{2}\right) \approx -(\alpha n + \beta n^2/2),$$

so no such correction is required. Making this change where appropriate places our point estimate of $\beta = 1.1 \times 10^{-4}$ approximately two orders of magnitude below those from other species, which range from 0.0074 in *E. coli* (Elena and Lenski 1997) to 0.014 in *A. niger* (average of two estimates, de Visser *et al.* 1997b) and 0.017 in foot-and-mouth disease virus (FMDV; Elena 1999). Our estimate of the degree of epistasis $\beta/\alpha = 0.0088$ is also lower than estimates from other species, which range from 0.039 in Aspergillus to 0.090 in FMDV and 0.14 in *E. coli*. Charlesworth (1998) calculates values of $\beta = 0.0027$ and $\beta/\alpha = 0.27$ for *D. melanogaster* and $\beta = 0.0011$ and $\beta/\alpha = 0.11$ for *D. pseudoobscura*, although these are indirect estimates and are not tested statistically. It is important to note that, as for the present result, in all cases where the epistasis effect has been tested statistically, it has been found to be nonsignificant (de Visser *et al.* 1997b; Elena and Lenski 1997; Elena 1999).

Significance for mutational hypotheses of sex and recombination: The mutational deterministic hypothesis for the maintenance of sexual reproduction (Kondrashov 1982, 1988; Charlesworth 1990; Kondrashov 1993) depends upon synergistic epistasis between deleterious mutations to increase the equilibrium mean fitness of sexual populations ($\overline{w}^*_{sex}$) relative to that of asexual populations ($\overline{w}^*_{asex}$). Whether or not a given degree of epistasis can provide an advantage to sex, however, depends upon the mutation rate. Charlesworth (1990) provides formulae for calculating mean fitnesses of large populations at equilibrium, given values of α_{het}, β_{het}, and the genomic deleterious mutation rate U. We may use these formulae with our estimates of β and α, assume that the dominance coefficient $h = 0.25$, and solve numerically for the value of U^*, the mutation rate per genome per generation that gives $\overline{w}^*_{sex} > 2\overline{w}^*_{asex}$ (*i.e.*, the point at which the sexuals overcome the twofold cost of sex).

This calculation also requires an assumption about the origin of the asexual lineage. It has been shown that an asexual population is expected to come into mutation-selection balance with mean fitness $\overline{w}^*_{asex} = e^{-U}$ (Kimura and Maruyama 1966), assuming that the originating clone had zero mutations. If the clonal population begins with $i > 0$ mutations, its mean fitness at equilibrium will be lower than e^{-U} (Charlesworth 1990). If clones originate only rarely from within a sexual population, then the minimum number of mutations likely to arise is two standard deviations less than the mean of the sexual population ($i = \overline{n} - 2\sigma$) (Charlesworth 1990). Alternatively, the clonal and sexual populations may begin with the same distribution of genotypes (*e.g.*, both originate at the same time from a source population); in this case, the asexual and sexual populations share the same minimum number of mutations, so effectively $i = 0$ and $\overline{w}^*_{asex} = e^{-U}$ (Kondrashov 1985; Charlesworth 1990).

If it is assumed that clonal populations begin with zero mutations (or with the same distribution as the sexual population; $i = 0$), then the mutation rate per genome per generation must be $U \geq \sim 2.8$ to maintain sexual reproduction under our point estimates of β and α, largely independent of the assumed number of mutations in each treatment (Table 6). In contrast, if clonal populations originate from within the distribution of sexual populations ($i = \overline{n} - 2\sigma$), mutation rates as low as $U = \sim 0.8$ can provide an advantage to sexual reproduction, and the advantage to sex actually increases as the degree of epistasis β/α decreases (Table 6). This counterintuitive pattern is not due to epistasis *per se*: It is a consequence of very weak selection against individual mutations—which can result from low α or from low β. When selection against mutations is weak, sexual populations come into equilibrium with large numbers of mutations—and clones originating from within those populations carry large numbers of mutations ($i \gg 0$). Those clones come into mutation-selection balance with very low fitness and are therefore easily outcompeted by the sexual population. This is a deterministic advantage to sexual reproduction that is based on deleterious mutations, but not dependent upon epistasis among mutations; to our knowledge it has not been pointed out previously. This mechanism has some similarities to Muller's ratchet (Muller 1964; Haigh 1978), in that it is driven by the inability of the asexual population to generate offspring with fewer mutations than its least-loaded genotype. It is also similar to the "Ruby in the Rubbish" mechanism, in which asexual populations are at a disadvantage because even advantageous mutations are locked into the genetic backgrounds in which they arose (Fisher 1930; Peck 1994). A potential problem with this argument is that it requires the asexual population to reach mutation-selection balance before the sexual population can overcome the twofold cost of sex. Unless population size is large, it is quite possible that the sexual population will be driven extinct before the asexual population reaches equilibrium (Charlesworth 1990; Howard 1994; Johnson 1999), in which case there can be no advantage to sex. However, the behavior of mutational models of sex under very weak selection coefficients has not been well explored, so the implications of these results for the maintenance of sex are not completely clear. In addition, even when mutational parameters (mutation rates, degree of epistasis) fall outside the range that allows mutations to maintain sexual reproduction on their own, they may interact with other factors (*e.g.*, host-parasite coevolution) to provide a net benefit to sex (Howard and Lively 1994, 1998; West *et al.* 1999).

For considerations of selection for recombination or for the frequency of sex within a sexual population, the situation appears to be at least slightly less complex. In this case, epistasis must be weak relative to the strength of selection for increased recombination to be favored, because strong synergistic epistasis causes an immediate reduction in fitness among recombinant offspring (Barton 1995; Charlesworth and Barton 1996). Since the strength of selection is determined by the mutation rate, this implies that if epistasis is weak, mutation rates can be relatively low and still cause selection for increased recombination (Charlesworth 1990; Barton 1995; Otto and Michalakis 1998). Thus, the existence of weak epistasis is quite favorable for the spread of an allele increasing recombination in a sexual population. This argument is counterbalanced, however, by the fact that variation in epistasis can decrease the advantage to recombination or remove it altogether (Otto and Feldman 1997). Although we cannot test for variation in epistasis, the three studies in which it has been examined have found variation in both the sign and magnitude of epistasis in bacteria and fungi (de Visser *et al.* 1997b; Elena and Lenski 1997; Whitlock and Bourguet 2000). In our case, with very weak epistasis, even

relatively low variance is likely to imply that many pairs of mutations interact with antagonistic, rather than synergistic, epistasis, and this may generate a net disadvantage to recombination.

We have shown that the net effect of epistasis on fitness is likely to be very low in *C. elegans*. These results add to the emerging picture that epistasis between deleterious mutations is very weak across a broad phylogenetic range (de Visser *et al.* 1997b; Elena and Lenski 1997; Charlesworth 1998; Elena 1999; but see Whitlock and Bourguet 2000). This suggests that mutation-selection balance is unlikely to be the sole factor maintaining sex: Either clones originate with zero mutations and the mutation rate must be very high to provide an advantage to sex or they originate from within the distribution of sexuals and are likely to drive the sexual population extinct before they reach mutation-selection balance. Similarly, the validity of the mutational model of recombination is likely to depend at least as much on variance in the degree of epistasis as it does on the mean, particularly when the mean is very low. Thus, it seems unlikely that selection against deleterious mutations alone can explain the ubiquity of sexual reproduction and recombination.

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