Low impact of germline transposition on the rate of mildly deleterious mutation in *Caenorhabditis elegans*

Mattieu Bégin and Daniel J. Schoen

Department of Biology, McGill University, Montréal, Québec, H3A 1B1, Canada.
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*Corresponding author:* Daniel J. Schoen, Department of Biology, McGill University, 1205 Dr. Penfield avenue, Montréal, Québec, H3A 1B1, Canada. E-mail: daniel.schoen@mcgill.ca, Phone: (514) 398-6461, Fax: (514) 398-5069.

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ABSTRACT

Little is known about the role of transposable element (TE) insertion in the production of mutations with mild effects on fitness, the class of mutations thought to be central to the evolution of many basic features of natural populations. We propagated mutation accumulation (MA) lines of two RNAi-deficient strains of *Caenorhabditis elegans* that exhibit germline transposition. We show here that the impact of TE activity was to raise the level of mildly deleterious mutation by 2- to 8.5-fold, as estimated from fecundity, longevity, and body length measurements, compared to that observed in a parallel MA experiment with a control strain characterized by a lack of germline transposition. Despite this increase, the rate of mildly deleterious mutation was between one and two orders of magnitude lower than the rate of TE accumulation, which was approximately two new insertions per genome per generation. This study suggests that high rates of TE activity do not necessarily translate into high rates of detectable non-lethal mutation.
INTRODUCTION

Transposable elements (TE) are DNA segments characterized by the ability to insert into new locations in the genome, potentially causing modifications to coding and regulatory regions, as well as chromosome breakage and genomic rearrangements. TE insertions can bring about major phenotypic effects and genetic diseases in plants and animals (McClintock 1953; Bingham et al. 1982; Kazazian et al. 1988), and are known to be responsible for a substantial fraction of mutations in many organisms (FinneGAN et al. 1978; Green 1988). While there have been a number of studies that examined the role of TE activity in quantitative genetic variation of certain phenotypes (e.g., Mackay 1985, 1987; Frankham et al. 1991; Torkamanzehi et al. 1992), little is known about the effect of TE insertions on the production of mutations with mildly deleterious effects on fitness, the class of mutations thought to be central to the evolution of many basic features of natural populations such as the mating system, recombination, standing genetic variation, and extinction of small demes (Lynch et al. 1999).

Some of the most detailed studies of spontaneous mild effect mutations have been conducted with the Bristol N2 natural strain of C. elegans (Keightley and Caballero 1997; Vassilieva and Lynch 1999; Vassilieva et al. 2000; Baer et al. 2005). Estimates of the rate of mildly deleterious mutation ($U$, inferred from phenotypic data) from these studies are among the lowest reported for eukaryotes, and have raised questions about the generality of results derived from classic mutation accumulation (MA) studies with Drosophila melanogaster (Mukai 1964). While transposition is known to occur in some
wild strains of *C. elegans*, it does not occur in the germline of N2 (Eide and Anderson 1985), a peculiarity hypothesized to be one of the principal reasons for the low deleterious mutation rate observed in this strain (Keightley and Caballero 1997; Vassilieva and Lynch 1999). It is of particular interest in this regard to address the potential contribution of TE activity to the rate of mildly deleterious mutation in *C. elegans*. For comparison with earlier work, it would be ideal to conduct any such investigation in the genetic background of the N2 strain. This is now possible due to the production of mutator strains of N2 (via EMS mutagenesis) deficient for gene products that play a role in the RNA interference (RNAi) pathway (Ketting *et al.* 1999; Tijsterman *et al.* 2002). In these mutants, the loss of function of genes underlying RNAi is associated with the activation in the germline of many TEs (Ketting *et al.* 1999), and it has been proposed, in fact, that RNA silencing is tantamount to the genome’s immune system with respect to the defence it provides against invading genetic elements (Plasterk 2000; Vasterhoudt and Plasterk 2004).

To explore the relationship between TEs and mildly deleterious mutations, we made use of the N2 strain as well as of two different RNAi mutant strains of *C. elegans*. We conducted MA experiments with each strain separately by maintaining single worm descent lines over many generations—a procedure that allows all but the most deleterious mutations to accumulate by genetic drift at a rate roughly equal to their rate of production (Keightley and Caballero 1997). In addition to examining changes in phenotypic measures related to fitness, we monitored the mobility and accumulation of three of the most active TEs (the DNA elements Tc1, Tc3 and Tc4) over the course of the experiments.
This enabled us to make comparisons of phenotypic estimates of mildly deleterious mutation among strains with different levels of TE activity.

MATERIALS AND METHODS

Worm maintenance and mutation accumulation (MA) protocol: The *Caenorhabditis elegans* strains, *mut-7* and *mut-14* (mutator strains), and Bristol N2, were obtained from the Caenorhabditis Genetics Center (Minneapolis, MN). Worms were maintained using a slightly modified standard protocol (STIERNAGLE 1999). For each strain, 75 replicate MA lines were initiated from a single homozygous progenitor (the product of twenty consecutive generations of self-fertilization) and independently propagated for 40 generations of MA by single worm descent (VASSILIEVA and LYNCH 1999). All surviving replicate lines where cryopreserved at generations 0, 20 and 40. The protocol used for worm culture was modified from STIERNAGLE (1999)—we used HB101 *E. coli* as a food source, kept worms at 20°, added 1mL/L of 200mg/mL streptomycin sulphate to both LB medium and NGM, used 20g/L of agar instead of 17g/L in the NGM, and used 10g/L of NaCL instead of 5g/L in the LB.

Transposon Display: Transposon Display (TD) is an AFLP-like strategy that allows detection of individual transposable elements (KORSWAGEN et al. 1996). With TD, nested transposon-specific PCR primers are used in combination with nested adaptor-specific primers—because the restriction sites lie at a range of different distances from the
transposon, the products visualized on the gel correspond to individual TE insertion positions.

We developed a modification of the TD procedure appropriate for small amounts of genomic DNA, as is available in single individuals of *C. elegans*. One to five worms were placed in 3 µL of worm lysis buffer (10 mM Tris-HCl pH 8.3, 2.5 mM MgCl₂, 50 mM KCl, 0.45% Tween 20, 0.45% Igepal, 0.01% gelatine, freshly added 60 µg/mL proteinase K) in 0.2 mL PCR tubes, frozen at −80° for at least one hour, then lysed by heating to 65° for 60 min, followed by 95° for 15 min. The transposon display protocol of KORSWAGEN et al. (1996) was adapted for use with the DNA sequencer IR² system (Li-Cor, Lincoln, NE). Single worm lysates were digested with 4 U NdeII (Promega, Madison, WI) and ligated overnight at 20° with 4 U T4 DNA ligase (Invitrogen, Burlington, ON, Canada) to a 15 µMol SAU3A adaptor cassette (KORSWAGEN et al. 1996). The digestion-ligation reaction product was diluted 4-fold and served as a template in PCR using an AmpliTaq PCR system (Applied Biosystems, Foster City, CA) in a PE Applied Biosystems GeneAmp 9700 thermocycler. The nested, adaptor-specific PCR primers, as well as the pre-selective and selective PCR primers for Tc1 and Tc4 are as given in KORSWAGEN et al. (1996). Pre-selective and selective PCR primers for Tc3 were: Tc3-2731: 5'-AACGAACGGCATTAGCAG and Tc3-2730: 5'-CGGAAGTTCTCCTAAACCTTC, respectively.
Pre-selective amplification was carried out with an element-specific primer and an adaptor-specific primer. The amplification products were diluted one hundred-fold before the selective PCR amplification, using nested element-specific primers and an IRDye-700 (Li-Cor) nested adaptor-specific primer. The reaction cycle for both amplifications consisted of 10 min at 94°; 20 cycles of 1 min at 94°, 1 min at 55°, and 1 min at 72°; and a final 10 min at 72°. The final amplification products were separated on a 41 cm, 5.5% denaturing polyacrylamide gel (BioShop, Burlington, ON, Canada) and were sized using the Li-Cor IRDye 50-700 sizing standard as a molecular weight marker.

To confirm that TD products correspond to transposon insertions, a sample of products were cloned with the TOPO-TA Cloning Kit (Invitrogen, Burlington, ON, Canada) and sequenced on a Li-Cor IR² DNA sequencer (or sent out for sequencing). These were separated on a silver stain sequencing gel (Promega, Madison, WI), and target bands were cut out of the gel and reamplified using the same primers and amplification conditions as for the selective PCR amplification above. The amplification products were then cloned and sequenced.

Several sets of TDs and subsequent cloning of products were conducted to validate the method. First, Tc1 elements were amplified by TD and cloned from single worm descent lines of N2 as described above. In this case, it is expected that all elements should correspond to known pre-existing insertions as present in the C. elegans genome database, as no germ line transposition is expected in the N2 lines. Secondly, Tc3 elements were amplified by TD and cloned from single worm descent lines of mut-7—in this experiment,
new insertions are expected. New insertions (those not present in the ancestor of the MA lines) were targeted for cloning.

In N2, sequenced amplification products all contained the Tc element expected plus the flanking region (data not shown). BLAST searches of the C. elegans genome database using as a query the sequence flanking the terminal inverted repeat (TIR) of the Tc element in question was carried out to confirm the genomic location. As expected in the case of N2, the flanking regions correspond to genomic regions with known Tc1 element sequences, whereas in the case of mut-7, the Tc3 elements are new insertions. Thus, both non-mobilized and recently mobilized Tc elements were isolated and identified by TD.

**Estimating the per generation rate of transposon accumulation:** The TD procedure was applied at generations 0 and 40 using separate single worm descent lines, comprising a random subset of the lines used for fitness assays. The number of mut-7 lines assayed for germline transposition of Tc1, Tc3, and Tc4 was 45, 40, and 44, respectively. In the case of mut-14, these numbers were 32, 31, and 30. We recorded the number of gel bands corresponding to each type of TE. Bands were distinct and numbered fewer than one hundred per lane (Figure 1). Taking into account the number of bands present per line at generations 0 and 40, and then fitting the change in number to a the dynamical model of transposition described below, allowed estimation of the rate of transposon accumulation by replicative and non-replicative transposition. These estimates of activity are conservative for three reasons. First, germline activity of one other TE, Tc5, has been reported in mut-7 (Ketting et al. 1999), but we were unable to consistently amplify Tc5
products, and so this element was not included in the present study. Second, TD bands smaller than 75 bp and larger than 500 bp could not be resolved well, and so were not included in the TE counts—based on the distribution of restriction site to element distances in N2, we would expect roughly two times as many bands if the entire gel region could be read accurately. Third, it is possible that some individual TD bands actually represent two or more products of the same size.

Let $T_{ijk}$ and $R_{ijk}$ denote the total and remaining number of insertions of element type $i$ in worm line $j$ at generation $k$—the term “remaining” denotes bands originally present at generation 0 that are still present at generation $k$. We note that band number may increase from one generation to the next if transposition is replicative, but the loss of bands originally present in an earlier generation is assumed to reflect the action of non-replicative transposition. While the assay procedure used in our work did not entail monitoring changes in the TD band patterns in every generation of single worm descent, it is useful to note that starting with an observed number of insertions of element type $i$ in line $j$, the total number of transposon insertions in future generations bears a simple relationship to the per element per generation rate of accumulation of insertions by replicative transposition ($u_r$). This relationship is:

$$T_{ij1} = T_{ij0} (1 + u_r),$$

$$T_{ij2} = T_{ij1} (1 + u_r) = T_{ij0} (1 + u_r)^2,$$

or, in general,

$$T_{ijk} = T_{ij0} (1 + u_r)^k$$

(1)
Equation (1) does not include a term for non-replicative transposition, since this mode of transposition contributes only to changing band positions and not the total number of insertions.

Continuing with this approach, we have:

\[ R_{ij1} = T_{ij0} (1 - u_n), \]

\[ R_{ij2} = T_{ij1} (1 - u_n) = T_{ij0} (1 - u_n)^2, \]

and, in general,

\[ R_{ijk} = T_{ij0} (1 - u_n)^k \]  (2)

where \( u_n \) is the per element per generation rate of accumulation of insertions by non-replicative transposition.

Finally, new insertions, those not present in the previous generation, could come about either from replicative or non-replicative transposition, and so we have:

\[ N_{ij1} = T_{ij0} (u_r + u_n), \]

\[ N_{ij2} = T_{ij1} (u_r + u_n) = T_{ij0} (1 + u_r) (u_r + u_n), \]

\[ N_{ij3} = T_{ij2} (u_r + u_n) = T_{ij0} (1 + u_r)^2(u_r + u_n), \]

and, in general,

\[ N_{ijk} = T_{ij0} (1 + u_r)^{k-1} (u_r + u_n) \]  (3)

All single worm descent lines within each transpositionally-active strain studied (mut-7 and mut-14) descend from a single inbred individual, and so the \( T_{ij0} \) values are identical for element type \( i \) and line \( j \). After propagation for 40 generations, however,
variable numbers of total ($T_{ij40}$), and remaining ($R_{ij40}$) insertions were observed for each line, as expected given the stochastic nature of transposition events (e.g., Figure 1; see Supplemental Table 1). Thus, the observations $T_{ij40}$ and $R_{ij40}$ together with equations (1) and (2) were used to obtain method-of-moment estimates of $u_r$ and $u_n$ for each line. Plotting $\sum N_{ijk}$ against $k$ yields a nearly linear relationship whose slope provides estimates of the per-line per generation rate of accumulation of new insertions.

**Fitness assays:** Lines were thawed prior to fitness assays and allowed to proliferate for 1 to 3 generations. Large numbers of eggs where then bleached, to remove *E. coli*, and the emerging larvae were synchronized (STIERNAGLE 1999). Three fitness components were measured on individual hermaphrodites: fecundity, longevity and body length. Efforts were made to randomize the measurement procedure with respect to the lines, but all the individuals of a line were always assayed on the same day and stacked together in the growth chamber. Fecundity was estimated by manually counting daily egg production of single adults, which involved transferring worms to a new plate containing *E. coli* every day, and then summing over the worm’s lifetime. Fecundity was statistically corrected for multiple observers by multiplying each observer’s counts by a factor determined through a test count of N2 controls. Fecundity is likely overestimated because some non-viable eggs (*i.e.*, eggs incapable of hatching) may be included in the counts. Longevity was scored as number of days that worms lived, and was determined by checking for the lack of body and pharynx movement. For body length, four days old worms were killed and preserved for at least a week in a non-toxic fixative made from 3mL of lactic acid, 1mL of acetic acid, 5mL of glycerol, 60mL of 100% ethanol, and 31mL of water. Length measurements were
performed on worms placed in multi-well micro-slides filled with fixative. The software programs QCapture v2.68.2 (QImaging, Burnaby, BC, Canada) and Image J v1.32 (National Institute of Health, Bethesda, MD) were used for image capture and measurement, respectively. Length data is reported in pixel numbers—1mm equals 1575.2 pixels.

Number of lines (worms) assayed for fecundity and longevity were 69 (683), 66 (637), and 51 (500) for N2, mut-7, and mut-14, respectively, and 29 (173), 30 (179), and 30 (178) lines for the controls. In the case of length, sample sizes were 63 (311), 62 (298) and 46 (212) for N2, mut-7, and mut-14, respectively, and 27 (133), 30 (141), and 28 (132) for the controls. For each MA line, 10 replicate worms per line were scored for fecundity and longevity, and 6 for body length. Control lines were created by randomly isolating individual worms from the generation 0 stock populations and, for each trait, 6 individual offspring within each line were randomly chosen as replicates. Assays were performed at generation 40 for N2 and at generation 20 for the mutator strains.

From the start of the experiment, strains differed phenotypically with respect to each trait (see Results). Additionally, the mean time between two consecutive generations (including restarting lines when they failed) for the N2, mut-7, and mut-14 strains was 3.3 d (s.d. = 0.6), 4.8 d (s.d. = 1.8), and 5.3 d (s.d. = 2.7), respectively, at generation 0. This initial difference likely reflects pleiotropic effects of RNAi deficiency, which have previously been reported to include temperature sensitivity, developmental abnormalities, and slower growth (Ketting et al. 1999; Hannon 2002; Wu-Scharf et al. 2000). While
the reduced survivability of the mutator strains renders long-term maintenance of MA lines
difficult, it does not confound the estimates of mildly deleterious mutation, which are based
on fitness trait changes that occur over generations within a strain.

**Mutation parameter estimation:** Least-squares means and among-line variance
components for fitness traits were estimated using the PROC GLM and VARCOMP-
REML, respectively, of SAS v9.1 (SAS Institute, Cary, NC). Standard errors were
estimated by the standard deviation of 1000 bootstrap replicates. When testing for the
equality of a parameter value across treatments (or with 0), statistical significance was
determined by checking whether the mean of a treatment was within 1.96*s.e. of the other
mean (or 0), a procedure adequate for roughly normal distributions. Estimation of the
haploid $U$, $a$ and $M_0$ were performed using the program MLGENOMEU v2.0818
(Keightley 1998). We programmed an automation of MLGENOMEU to calculate
bootstrap distributions of $Ua / M_0$ % (see Results), and used the median absolute deviation
about the median (MAD) as a robust estimate of the standard deviation because the
distribution had long tails of outliers (SAS INSTITUTE 2003). Values for N2-body length
were estimated by constraining all mutations to have positive effects, because the mean of
that trait increased during MA in most lines. We attempted to estimate the proportion of
positive mutations but whenever this proportion approached 50%, as it often did in the case
of N2-body length, the estimates of $U$ and $a$ were unstable.

Though it is a less statistically efficient method, we also estimated mutation
parameters via the Bateman-Mukai method (see Supplemental Table 2), for comparison
with earlier MA studies. The Bateman-Mukai formulas (Lynch and Walsh 1998) were applied directly to our estimates of mean trait values and among-line variance components. The estimation of mutational parameters (both by MLGENOMEU and the Bateman-Mukai method) could be biased because all the individuals of a MA line were reared in the same plate, and thus common environmental effects are confounded with line effects. However, it is reasonable to assume that common environmental effects do not change drastically between the beginning and end of the MA experiments, and thus the confounding effect of this factor should be minimal.

RESULTS

Transposition rates: Transposon display (TD) was used to detect new TE insertions and quantify levels of transposition in each of the MA experiments described above. As expected (Eide and Anderson 1985), no new insertions were detected in any of the N2 lines during the course of 40 generations of MA, but TD patterns revealed that substantial element mobility occurred in the mutator strains over this same period (Figure 1, Table 1). Estimated rates of accumulation of transposons in the mutator strains by replicative transposition were on the order of 0.01 transposition events per element per generation, about five- to ten-fold higher than the estimated rate of non-replicative transposition (Table 1, see Supplemental Table 1). In the mut-7 strain, Tc4 elements were the most active TEs, while in the mut-14 strain, Tc3 elements showed the highest activity levels. The combined rate of accumulation of new Tc1, Tc3, and Tc4 insertions was approximately the same in
the two mutator strains, averaging 1.91 element insertions per generation in MA lines of the
mut-7 strain, and 1.98 in mut-14 (Table 1). These estimates of transposon activity are likely
to be conservative (see Materials and Methods).

**Fitness assays:** Assays of fecundity, longevity, and body length were conducted with
control lines (generation 0) and MA lines. The mut-7 and mut-14 strains exhibited a
reduction in the mean of all measured fitness traits (the reduction was not always
significant), and a significant increase in the among-line component of variance ($V_L$) for
these traits, as expected when deleterious mutations accumulate independently in MA lines
(Table 2). In the N2 strains, length increased (N.S.) after MA whereas the two other traits
decreased significantly, and $V_L$ increased significantly only for longevity and length (Table
2).

**Mutation parameters:** Studies of mildly deleterious mutations typically use phenotypic
data to estimate the haploid genomic mutation rate ($U$) for fitness components and the
average homozygous effect of individual mutations ($a$). Joint estimation of these two
parameters is often unreliable, however, because negative correlation between $U$ and $a$ is
expected (i.e., lines may decline in fitness either because of many mutations of small effect
or few mutations of large effect). This was indeed the case with our data set for each
combination of fitness components and strains ($r$ ranged from -0.26 to -0.87, all $P < 0.001$,
Figure 2A). The parameter $Ua / M_0 \%$, the standardized per-generation rate of change in the
mean of a fitness component, is a more robust parameter because it circumvents the
potential confounding of $U$ and $a$, and allows comparisons across strains or species.
For each fitness component, all estimates of $U, a$ and $Ua / M_0 \%$ were significant (Table 3). The parameter $Ua / M_0 \%$ was 2 to 8.5 times larger in mut-7 and mut-14 compared with N2 (Figure 2B-2D, Table 3). All differences were significant except in the case of longevity between mut-14 and N2 (Figure 2C, Table 3). The difference in $Ua / M_0 \%$ between the mutator strains and N2 corresponds to the effect of TE activity. Altering the assumed distributions of mutational effects (i.e. using different values of $\beta$, the shape parameter of the $\gamma$ distribution of mutational effects (KEIGHTLEY and ONISHI 1998)) did not substantially alter the estimation of $Ua / M_0 \%$ (Figure 2B-2D, see Supplemental Table 3), and similar results were obtained for the two different mutator strains, suggesting that the estimation of the difference in $Ua / M_0 \%$ between the N2 and mutator strains is robust.

There was no significant correlation between the number of new fixed TE insertions per line and the line value of fitness components ($r$ ranged from -0.28 to 0.31, all N.S.). Although this is not a genetic correlation, because it also contains common environmental effects, the symmetrical distribution of the correlations around 0 suggests no bias.

DISCUSSION

Transposition rates: The rates of transposition observed in this study are mostly of magnitude $10^{-2}$ insertions per element per generation and, therefore, are at the high end of the spectrum of reported transposition rates in eukaryotic genomes. For instance, direct
estimates of transposition in Drosophila, based on in situ hybridization studies in inbred lines, are of the magnitude $10^{-3}$ to $10^{-4}$ insertions per element per generation, and in yeast are of magnitude $10^{-5}$, though unlike the elements investigated here, these estimated rates are for retrotransposons (Harada et al. 1990; Curcio and Garfinkel 1991; Nuzhdin and Mackay 1994). Transposition rates approaching or exceeding the magnitude observed in this study have, however, been reported in Drosophila crosses for P elements as well as retrotransposons (Berg and Spradling 1991; Labrador et al. 1999).

**Deleterious mutation rates:** The inconsistency across traits of the decrease in mean trait value and increase in $V_L$ observed in N2 is not unlike the results of other N2 studies that used a similar number of MA generations, and is consistent with the low rate of deleterious mutation estimated in this strain (Keightley and Caballero 1997; Vassilieva and Lynch 1999). Likewise, the estimates of the per-generation rate of decline in trait value $(Ua / M_0 \%)$ in N2 fall within the range reported in other published studies: 0.03% to 0.28% for fecundity, 0.02% to 0.31% for longevity, and 0.02% to 0.23% for various measures of body size (Keightley and Caballero 1997; Vassilieva and Lynch 1999; Vassilieva et al. 2000; Azevedo et al. 2002; Ajie et al. 2005; Baer et al. 2005). The estimates of $Ua / M_0 \%$ for the mutator strains are all larger than those of N2, more so in fecundity than in the two other traits studied, which seems to reflect the higher mutational input caused by TEs.

**From genotype to phenotype:** An expected rate of deleterious mutation corresponding to the activity of TEs may be calculated by combining information on the estimated per-generation numbers of TE insertions in MA lines with information on TE insertion site
distribution. The latter results were obtained by cloning and sequencing of new TE insertions in other studies using the *mut-7* strain (MARTIN et al. 2002; VAN DER LINDEN and PLASTERK 2004), and revealed that 12-20% of newly arising Tc1 insertions were in exons and 5-19% in the short upstream or downstream regions adjacent to coding sequences (with the remainder transposing into introns or intergenic regions). Taking the figure of 12% insertions into exons as characteristic of all TE insertions studied here (i.e., Tc1, Tc3, and Tc4), and assuming that all such insertions are deleterious, the total expected rate of deleterious mutation per generation is 0.23 for *mut-7* and 0.24 for *mut-14*. This is likely to be a low estimate because it uses the lower bound figure for insertion into exons, it excludes possible deleterious effects of TE insertion into regulatory regions of the genome, and because our estimates of transposition rates are conservative (see Materials and methods). As well, the nearly homozygous lines used in this work offer little opportunity for chromosomal rearrangements through TE ectopic exchange, which may further reduce the deleterious fitness effects of TEs (CHARLESWORTH and CHARLESWORTH 1995).

While larger than those we observed in N2, the haploid deleterious mutation rates estimated in the mutator strains (which include mutations caused by TE as well as all other sources of spontaneous mutations) were lower than the expected rates of deleterious mutation caused solely by accumulation of new TE insertions—the difference being more than one order of magnitude when all sources of underestimation of the transposition rate are considered (see above). Moreover, a lack of association between germline activity of TEs and rates of mild effect mutation is seen at the individual line level within each mutator strain. Taken together, these results suggest that the majority of TE insertions monitored in
this study, though they may contribute to the pool of mildly deleterious mutations that is central to the evolution of populations, are undetectable, likely because of their individually small, almost neutral, effects. In fact, this type of result has also been seen elsewhere with *C. elegans*—MA studies using direct sequencing to compare rates of spontaneous mutation with actual genomic rates of mutation (DENVER *et al*. 2004), as well as studies that employed EMS mutagenesis (DAVIES *et al*. 1999) or mismatch repair defects (ESTES *et al*. 2004) to compare observed and theoretically expected mutation rates have converged on the observation that only about one out of a hundred non-lethal mutations are detectable phenotypically. This suggests the extent to which developmental pathways and phenotypes in this species are canalized, or robust in the face of genetic variation. In the case of transposition, splicing of TE insertions from mRNA may contribute to robustness. In the N2 strain, RUSHFORTH and ANDERSON (1996) showed that, in alleles of two muscle genes in which a Tc1 insertion was present either in introns or exons, splicing often removed the TE, producing a wild type phenotype. This mechanism may occur with all types of TEs studied here (RUSHFORTH and ANDERSON 1996) and, if generally applicable to most genes, could explain the discrepancy between expected and observed deleterious mutation rates.

**Variation in the deleterious mutation rate across species:** Our estimates of per-generation decline in mean fitness (best represented by fecundity) in the *C. elegans* strains that exhibit germline transposition are similar or higher than classical (MUKAI 1964) and recent (e.g. CHARLESWORTH *et al*. 2004) estimates reported for *D. melanogaster*. This suggests that lack of transposition in N2 could account, in part, for the observed discrepancy between the two taxa. However, differences exist, most notably with respect to
the haploid deleterious mutation rate, which is lower in the mutator strains of *C. elegans* than in Drosophila, despite high rates of transposition. Moreover, the observed absence of a correlation between number of TE insertions and fitness decline in mutator strains of *C. elegans* contrasts with the significantly negative correlation reported in Drosophila (Houle and Nuzhdin 2004; Pasynkova et al. 2004; the latter, however, includes the effect of lethal mutations), and with previous experimental demonstrations that TE insertions cause significant fitness decline in Drosophila (Eanes et al. 1988; Lyman et al. 1996) or *Escherichia coli* (Elena and Lenski 1997). These discrepancies may be explained by the differences in the type and effect of active TEs across species (*copia, P, roo*, and *Doc* elements are active in Drosophila), and by other classes of spontaneous mutations that also differ in frequency of occurrence (Keightley and Charlesworth 2004). The results of a comparison of mutation rate and effect across a total of six strains taken from three species of Rhabditid nematodes show that there is genetic variation in mutational properties at these two taxonomic levels (Baer et al. 2005). These combined results lend credence to the view that rates of mild effect mutation are determined by various factors that vary across species.
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LITERATURE CITED


Transposon Tc1-derived, sequence-tagged sites in *Caenorhabditis elegans* as markers

LABRADOR, M., M. FARRE, F. UTZET, and A. FONTDEVILA, 1999 Interspecific hybridization

LYMAN, R. F., F. LAWRENCE, S. V. NUZHDIN, and T. F. C. MACKAY, 1996 Effects of single
*P*-element insertions on bristle number and viability in *Drosophila melanogaster*.
Genetics **143**: 277-292.

LYNCH, M., and B. WALSH, 1998 *Genetics and Analysis of Quantitative Traits*. Sinauer
Associates, Sunderland, MA.

LYNCH, M., J. BLANCHARD, D. HOULE, T. KIBOTA, S. SCHULTZ *et al*., 1999 Spontaneous
deleterious mutation. Evolution **53**: 645-663.

MACKAY, T. F. C., 1985 Transposable element-induced response to artificial selection in
*Drosophila melanogaster*. Genetics **111**: 351-374.

MACKAY, T. F. C., 1987 Transposable element-induced polygenic mutations in *Drosophila

MARTIN, E., H. LALOUX, G. COUETTE, T. ALVAREZ, C. BESSOU *et al*., 2002 Identification
of 1088 new transposon insertions of *Caenorhabditis elegans*: a pilot study toward
large-scale screens. Genetics **162**: 521-524.

MCCLINTOCK, B., 1953 Induction of instability at selected loci in maize. Genetics **38**: 579-
599.

MUKAI, T., 1964 The genetic structure of natural populations of *Drosophila melanogaster*.
PASYUKOVA, E. G., S. V. NUZHDIN, T. V. MOROZOVA, and T. F. C. MACKAY, 2004

Accumulation of transposable elements in the genome of Drosophila melanogaster is associated with a decrease in fitness. J. Hered. 95: 284-290.


SAS INSTITUTE, 2003 SAS 9.1.3 Help and Documentation. SAS Institute, Cary, NC.


**TABLE 1**

Transposition in MA lines of two mutator strains of *C. elegans*

<table>
<thead>
<tr>
<th>Strain/element</th>
<th>( T_{ij0} )</th>
<th>( T_{ij40} ) (s.d.)</th>
<th>( R_{ij40} ) (s.d.)</th>
<th>( \bar{u}_r ) (s.d.)</th>
<th>( \bar{u}_n ) (s.d.)</th>
<th>Mean per line rate (s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>mut-7</em>/Tc1</td>
<td>32</td>
<td>49.6 (5.4)</td>
<td>29.4 (1.6)</td>
<td>0.011 (0.003),</td>
<td>0.002 (0.001)</td>
<td>0.525 (0.150)</td>
</tr>
<tr>
<td><em>mut-7</em>/Tc3</td>
<td>34</td>
<td>39.6 (6.1)</td>
<td>32.0 (2.0)</td>
<td>0.010 (0.004),</td>
<td>0.002 (0.002)</td>
<td>0.407 (0.182)</td>
</tr>
<tr>
<td><em>mut-7</em>/Tc4</td>
<td>29</td>
<td>62.8 (11.5)</td>
<td>27.0 (1.5)</td>
<td>0.021 (0.004),</td>
<td>0.002 (0.002)</td>
<td>0.977 (0.297)</td>
</tr>
<tr>
<td><em>mut-14</em>/Tc1</td>
<td>32</td>
<td>36.7 (2.2)</td>
<td>31.3 (0.7)</td>
<td>0.003 (0.002),</td>
<td>0.001 (0.001)</td>
<td>0.136 (0.056)</td>
</tr>
<tr>
<td><em>mut-14</em>/Tc3</td>
<td>26</td>
<td>77.3 (10.5)</td>
<td>20.5 (3.0)</td>
<td>0.021 (0.004),</td>
<td>0.005 (0.003)</td>
<td>1.315 (0.287)</td>
</tr>
<tr>
<td><em>mut-14</em>/Tc4</td>
<td>27</td>
<td>48.8 (9.9)</td>
<td>26.0 (1.4)</td>
<td>0.013 (0.005),</td>
<td>0.001 (0.001)</td>
<td>0.538 (0.247)</td>
</tr>
</tbody>
</table>

\( T_{ij0} \): number of TE insertions per line at generation 0 (uniform for all lines). \( T_{ij40} \): mean number of TE insertions per line at generation 40. \( R_{ij40} \): mean number of original TE insertions per line remaining by generation 40. \( \bar{u}_r \) and \( \bar{u}_n \): mean per element per generation rates of transposon accumulation by replicative and non-replicative transposition, respectively. Mean per line rate: mean number of new insertions per line per generation by replicative and non-replicative transposition combined.
TABLE 2

Estimates of the mean trait values and among-line variances (standard error) for control and MA lines of three *C. elegans* strains

<table>
<thead>
<tr>
<th>Trait</th>
<th>Parameter</th>
<th>N2</th>
<th>mut-7</th>
<th>mut-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity</td>
<td>$M_0$</td>
<td>290.2 (10.0)</td>
<td>101.5 (7.6)</td>
<td>99.6 (5.7)</td>
</tr>
<tr>
<td></td>
<td>$M_{MA}$</td>
<td>273.6 (5.2)</td>
<td>85.0 (5.1)</td>
<td>74.4 (4.7)</td>
</tr>
<tr>
<td></td>
<td>$V_{L:0}$</td>
<td>1097.1 (344.9)</td>
<td>342.7 (198.5)</td>
<td>254.8 (110.0)</td>
</tr>
<tr>
<td></td>
<td>$V_{L:MA}$</td>
<td>1521.5 (277.2)</td>
<td>1490.5 (232.5)</td>
<td>944.5 (165.7)</td>
</tr>
<tr>
<td>Longevity</td>
<td>$M_0$</td>
<td>20.8 (0.7)</td>
<td>20.0 (0.8)</td>
<td>15.7 (0.5)</td>
</tr>
<tr>
<td></td>
<td>$M_{MA}$</td>
<td>19.0 (0.4)</td>
<td>18.4 (0.6)</td>
<td>14.2 (0.4)</td>
</tr>
<tr>
<td></td>
<td>$V_{L:0}$</td>
<td>0*</td>
<td>0*</td>
<td>0*</td>
</tr>
<tr>
<td></td>
<td>$V_{L:MA}$</td>
<td>4.2 (1.2)</td>
<td>14.6 (3.5)</td>
<td>3.5 (1.1)</td>
</tr>
<tr>
<td>Length</td>
<td>$M_0$</td>
<td>2107.0 (22.7)</td>
<td>1801.4 (36.2)</td>
<td>1840.0 (34.8)</td>
</tr>
<tr>
<td></td>
<td>$M_{MA}$</td>
<td>2251.4 (15.4)</td>
<td>1764.5 (28.2)</td>
<td>1796.7 (36.3)</td>
</tr>
<tr>
<td></td>
<td>$V_{L:0}$</td>
<td>2981.0 (1684.5)</td>
<td>13421.2 (3160.3)</td>
<td>6082.0 (2498.7)</td>
</tr>
<tr>
<td></td>
<td>$V_{L:MA}$</td>
<td>12876.2 (2890.8)</td>
<td>43832.3 (7580.5)</td>
<td>57085.4 (14895.5)</td>
</tr>
</tbody>
</table>

$M_0$: mean trait value at generation 0 (control). $M_{MA}$: mean trait value after mutation accumulation. $V_{L:0}$: among-line variance component at generation 0 (control). $V_{L:MA}$: among-line variance component after mutation accumulation. Parameters were estimated using the SAS program and standard errors were obtained through bootstrapping (see Materials and methods). *: no standard error is provided (between 62% and 94% of the bootstrap estimates were 0).
TABLE 3

Estimates of mutation parameters (standard error), assuming equal mutational effects, in three *C. elegans* strains

<table>
<thead>
<tr>
<th>Trait</th>
<th>Parameter</th>
<th>N2</th>
<th>mut-7</th>
<th>mut-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity</td>
<td>$U$</td>
<td>0.008 (0.002)</td>
<td>0.026 (0.005)</td>
<td>0.070 (0.019)</td>
</tr>
<tr>
<td></td>
<td>$a$</td>
<td>-68.0 (6.7)</td>
<td>-61.0 (10.6)</td>
<td>-25.2 (6.1)</td>
</tr>
<tr>
<td></td>
<td>$Ua/M_0%$</td>
<td>-0.19 (0.04)</td>
<td>-1.4 (0.2)</td>
<td>-1.6 (0.2)</td>
</tr>
<tr>
<td>Longevity</td>
<td>$U$</td>
<td>0.026 (0.013)</td>
<td>0.039 (0.012)</td>
<td>0.031 (0.015)</td>
</tr>
<tr>
<td></td>
<td>$a$</td>
<td>-1.7 (0.6)</td>
<td>-3.8 (0.8)</td>
<td>-2.3 (0.7)</td>
</tr>
<tr>
<td></td>
<td>$Ua/M_0%$</td>
<td>-0.21 (0.06)</td>
<td>-0.69 (0.12)</td>
<td>-0.46 (0.13)</td>
</tr>
<tr>
<td>Length</td>
<td>$U$</td>
<td>0.035 (0.008)</td>
<td>0.033 (0.006)</td>
<td>0.056 (0.012)</td>
</tr>
<tr>
<td></td>
<td>$a$</td>
<td>78.9 (15.6)</td>
<td>-222.8 (19.2)</td>
<td>-171.0 (16.8)</td>
</tr>
<tr>
<td></td>
<td>$Ua/M_0%$</td>
<td>0.13 (0.02)</td>
<td>-0.39 (0.06)</td>
<td>-0.49 (0.09)</td>
</tr>
</tbody>
</table>

$U$: haploid mutation rate. $a$: average homozygous mutational effect. $M_0$: mean trait value at generation 0 (control). $Ua/M_0\%$: standardized per-generation rate of change in mean trait value, expressed as a percentage. These parameters were all estimated using the program MLGENOMEU ($\beta \rightarrow \infty$, see Material and methods). Values for N2-length were estimated by constraining all mutations to have positive effects because the mean of that trait increased during MA in most lines.
FIGURE 1.—Sample TD gels showing Tc3 insertions in a subset of lines from separate MA experiments with *C. elegans* strains N2, *mut*-7, and *mut*-14. Band patterns shown at generation 0 (control) and after 40 generations of MA. Size standard (in bp) is shown on the left.

FIGURE 2.—Mutation parameter estimates for the three *C. elegans* MA studies. (A) Typical empirical bivariate bootstrap distribution of the mutation parameters $U$ and $a$ for the trait fecundity in *mut*-14. Parameter values were obtained using MLGENOMEU with the constraint of equal mutational effects ($\beta \to \infty$). Each of the 1000 data points corresponds to a bootstrap resampling of the original data set. (B-D) Comparison, among strains and traits, of the absolute values of $Ua / M_0$, the standardized per-generation rate of change in the mean of a fitness component, expressed as a percentage. (B) fecundity (lifetime egg production) (C) longevity (number of days lived), (D) body length (pixels). Parameter values were obtained using MLGENOMEU and are shown for 2 mutational effect distributions: $\beta \to \infty$ (●) and $\beta = 1$ (■). The value for N2-length was estimated by constraining all mutations to have positive effects because the mean of that trait increased during MA in most lines. Error bars represent ± 1 standard error. All $Ua / M_0$ estimates significantly differ from 0.