Increased oxidative damage to DNA in the lab environment cannot explain why the *C. elegans* mutation spectrum is different in the lab and in nature.

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Background/Introduction

The rate and spectrum of mutation are of fundamental importance in evolutionary biology. Mutation accumulation (MA) experiments are the usual (and always the most efficient) way to estimate the properties of spontaneous mutation divorced from the influence of natural selection. It is now apparent that the base-substitution spectrum of mutations accumulated in lab MA populations of *C. elegans* differ significantly and consistently from the standing site-frequency spectrum (SFS) in nature, with a greater proportion of transversions in the lab, especially C:G→A:T transversions. One possible explanation for the discrepancy is that conditions in the lab result in increased oxidative damage to DNA relative to that in nature, perhaps associated with differences in metabolism (e.g., food ad libitum). To test that hypothesis, we performed an MA experiment with a mutant strain of *C. elegans*, mev-1, that is known to experience elevated steady-state oxidative stress, resulting from a defective complex II of the mitochondrial electron transport chain. If oxidative stress is a cause of the difference, we expect an even greater skew toward C:G→A:T transversions, which are a signature of oxidative damage to DNA.

Mev-1 Gene

Mev-1 gene, located on chromosome III, has a mitochondrial electron transfer activity. Mev-1 encodes a subunit of the enzyme succinate dehydrogenase cytochrome b, which is a component of complex II of the mitochondrial electron transport chain (Ishii, T. et al., 1998). The mev-1(kn1) mutant decreases the ability of complex II to catalyze electron transport, which results in increased mitochondrial reactive oxygen species (ROS) production, resulting in increased oxidative stress (Ishii, N. et al., 1998) that is potentially mutagenic (Ishii, T. et al., 2005). The mev-1 mutant generation is explained in Joyner-Matos et al (2011). Briefly, by backcrossing the mev-1(kn1) allele into the N2 genomic background for 12 generations, followed by six generations of selfing, the mutant strain is ready for the next step.

MA experiment

The mutation accumulation experiment was done using standard *C.elegans* MA procedures (Vassilieva & Lynch, 1999). Briefly, worms of the N2, PB306, and mev-1 strains were allowed to grow to a large population size. 100 replicates lines of N2 and PB306 and 72 replicate lines of mev-1 strain were allowed to evolve under relaxed selection for ~ 250 generations for N2 and PB306 and ~125 generations for mev-1, at which point the surviving lines were cryopreserved. Details of the N2 and PB306 lines are reported in Baer et al. (2005); details of the mev-1 lines are reported in Joyner-Matos et al. (2011).
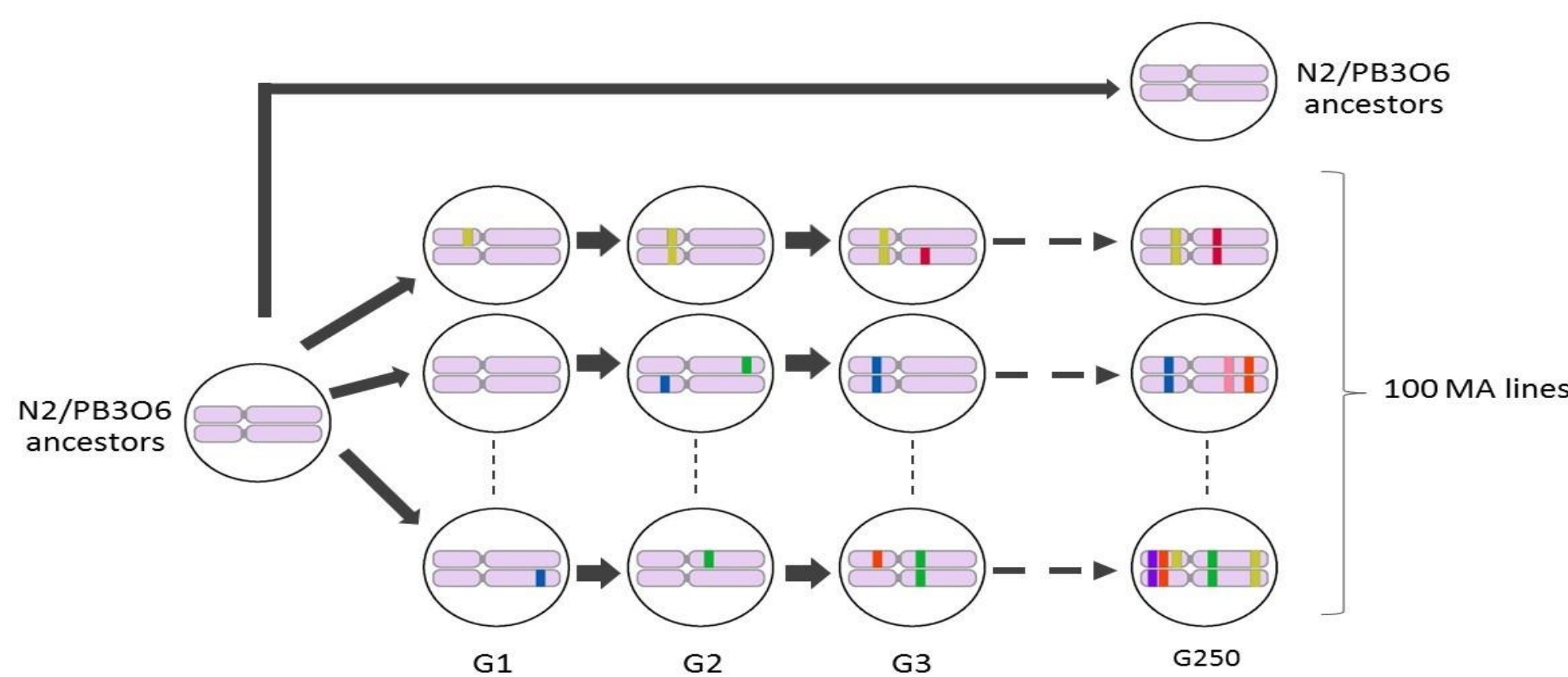
Figure 1. Schematic diagram of the MA experiment in *C. elegans*. Mutations represented as colored blocks within chromosomes. G250 refers to Generation 250. The G max for mev-1 MA line is 125.

Whole genome sequencing

- **Whole-Genome Sequencing:** using Illumina short-read sequencing at an average coverage of ~ 27X depth with 100 bp paired-end reads.

- **Assembly of the genome:** Trimming the raw sequencing reads was done using fastp (Chen, Zhou, Chen, & Gu, 2018). We used bowtie2 to align trimmed sequence data to the N2 reference genome (WS263). Duplicate reads will be identified and removed using MarkDuplicates tool in Genome Analysis Toolkit (GATK)/picard.

- **Variant calling:** We used HaplotypeCaller in GATK4 (McKenna et al., 2010) for variant calling.

- Identification of variants: We will include only the homozygous variants that are present in one and only one MA lines for each datastore.

- **Mutation Rate Calculation:** The mutation rate for each MA line was calculated using this formula:

  $$\mu_i = (X_i)/(D_i)(G_i)$$

- where X_i is the total number of SNPs/indels for each MA line, D_i is the detectable site of each MA line and G_i is the number of generations for each MA line in the MA experiment.

- **Mutation Spectrum:** Mutation rate for six base-substitution types for each MA lines was estimated by using a number of G:C and A:T sites for each mutation type and the average rate for N2 MA lines and PB306 MA lines was compared with mev-1 MA lines.
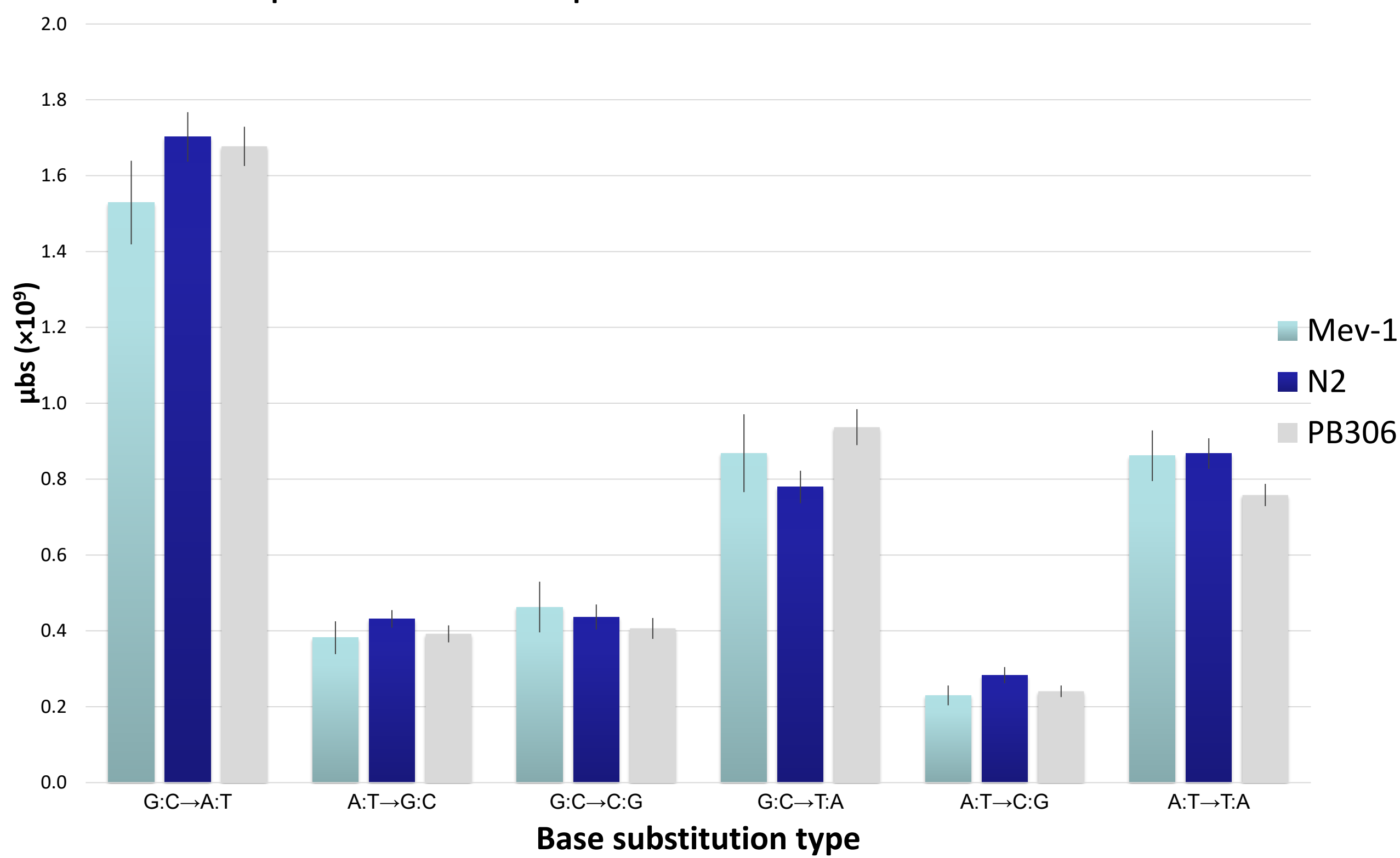
Mutation rate estimation

- The total mutation rate of mev-1 lines does not differ from N2 lines (t-test, P=0.96) and PB306 lines (t-test, P=0.68). Also, the total mutation rate of N2 lines does not differ from PB306 lines(t-test, P=0.59).

- The base substitution rate of mev-1 lines does not differ from N2 lines (t-test, P=0.06) and PB306 lines (t-test, P=0.99).

- The average Transition/Transversion ratio for mev-1 lines was 0.67, For N2 MA lines 0.75 and for PB306 MA lines 0.76.

- The Ts/Tv ratio does not differ significantly between mev-1 MA lines and N2 MA lines (T-test, P=0.63), between mev-1 MA and PB306 MA lines (T-test, P=0.65) and between N2 and PB306 MA lines (T-test, P=0.92).

- The Ts/Tv ratio of rare variants and common variants in wild isolates was 1.25 and 1.27, respectively.

Table 1. Average Mutation Rates and transition to transversion ratio.

	Mev-1	N2	PB306
μ_{bs} (×10^9)	1.97	2.05	1.97
μ_{INDEL} (×10^9)	0.77	0.60	0.73
μ_{Total} (×10^9)	2.74	2.66	2.71
Ts/Tv ratio	0.67	0.75	0.76

Mutation was biased towards A/T

- There was a bias toward G:C→A:T transitions among all three strains.

- There was an excess from G:C→A:T mutations over A:T→G:C mutations for all three strains which show departure from equilibrium G+C content.

Figure 2. Base substitution rate estimates for the six base substitution types. Error bars show standard error of the mean approximations.

Table 2. P-value of base substitution rate estimates for the six base substitution types.

	G:C→A:T	A:T→G:C	G:C→C:G	G:C→T:A	A:T→C:G	A:T→T:A
N2-PB306	0.766	0.223	0.487	0.015	0.108	0.030
Mev-1-N2	0.185	0.317	0.728	0.430	0.121	0.942
Mev-1-PB306	0.233	0.836	0.443	0.548	0.721	0.165

Discussion/Conclusion

Whole-genome sequencing of mev-1 MA lines that had accumulated mutations for >100 generations revealed a rate and spectrum of mutations that are essentially identical to other *C. elegans* MA lines. Thus, there is no evidence that the discrepancy between the lab-accumulated and natural spectra is the result of increased oxidative damage under lab conditions. Oxidative stress is known to increase the somatic mutation rate; apparently the germline is uniquely protected against oxidative damage.

Several lines of evidence show that oxidative damage to DNA causes two types of mutation more frequently. First, the major source of the G:C→T:A transversions is believed to be 8-oxoguanine and second, G:C→A:T transition (5-Hydroxyuracil) which also results from oxidative damage to DNA. If oxidative stress is a cause of the difference, we expect an even greater skew toward G:C→T:A transversions and G:C→A:T transition which are a signature of oxidative damage to DNA, while in our study there was no difference between G:C→T:A transversions and G:C→A:T transition of mev-1-derived MA lines with N2-derived MA lines and PB306-derived MA lines.

In an attempt to see if the oxidative damage to DNA account for a significant source of germline mutations, Joyner-Matos et al (2011) did an experiment with the same mev-1 MA lines (Joyner-Matos et al, 2011). They reported that there is no difference between the mutational decline in fitness for mev-1 MA lines and N2 MA lines. Given their result, we can conclude that there is no evidence that the genomic mutation rate differs between mev-1 and N2 MA lines.

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