Low Base-Substitution Mutation Rate in the Germline Genome of the Ciliate *Tetrahymena thermophila*

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Abstract

Mutation is the ultimate source of all genetic variation and is, therefore, central to evolutionary change. Previous work on *Paramecium tetraurelia* found an unusually low germline base-substitution mutation rate in this ciliate. Here, we tested the generality of this result among ciliates using *Tetrahymena thermophila*. We sequenced the genomes of 10 lines of *T. thermophila* that had each undergone approximately 1,000 generations of mutation accumulation (MA). We applied an existing mutation-calling pipeline and developed a new probabilistic mutation detection approach that directly models the design of an MA experiment and accommodates the noise introduced by mismapped reads. Our probabilistic mutation-calling method provides a straightforward way of estimating the number of sites at which a mutation could have been called if one was present, providing the denominator for our mutation rate calculations. From these methods, we find that *T. thermophila* has a germline base-substitution mutation rate of 7.61×10^{-12} per-site, per cell division, which is consistent with the low base-substitution mutation rate in *P. tetraurelia*. Over the course of the evolution experiment, genomic exclusion lines derived from the MA lines experienced a fitness decline that cannot be accounted for by germline base-substitution mutations alone, suggesting that other genetic or epigenetic factors must be involved. Because selection can only operate to reduce mutation rates based upon the "visible" mutational load, asexual reproduction with a transcriptionally silent germline may allow ciliates to evolve extremely low germline mutation rates.

Key words: drift-barrier hypothesis, mutation accumulation, micronucleus, macronucleus, microbial eukaryote, Oligohymenophorea.

Introduction

Mutation is the ultimate source of all genetic variation, and the rate, molecular spectrum, and phenotypic consequences of new mutations are all important drivers of biological processes such as adaptation, the evolution of sex, the maintenance of genetic variation, aging, and cancer. However, because mutations are rare, detecting them is difficult, often requiring the comparison of genotypes that have diverged from a common ancestor by at least hundreds or thousands of generations. Further, interpreting the results of such comparisons is complicated by the fact that mutations are frequently eliminated by natural selection before they can be studied.

Mutation accumulation (MA) is a standard method for studying mutations experimentally. In a typical MA experiment, many inbred or clonal lines are isolated and passed repeatedly through bottlenecks. This reduces the effective population size and lessens the efficiency of selection, allowing all but the most deleterious mutations to drift to fixation (Bateman 1959; Mukai 1964). The genome-wide mutation rate and mutational spectrum can then be estimated by comparing the genomes of MA lines with those of their ancestors.