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Evolution

Muller's ratchet decreases fitness of a DNA-based microbe

(mutation rate/*Salmonella typhimurium*)

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ABSTRACT **Muller proposed that an asexual organism will inevitably accumulate deleterious mutations, resulting in an increase of the mutational load and an inexorable, ratchet-like, loss of the least mutated class [Muller, H. J. (1964) *Mutat. Res.* 1, 2–9]. The operation of Muller's ratchet on real populations has been experimentally demonstrated only in RNA viruses. However, these cases are exceptional in that the mutation rates of the RNA viruses are extremely high. We have examined whether Muller's ratchet operates in *Salmonella typhimurium*, a DNA-based organism with a more typical genomic mutation rate. Cells were grown asexually under conditions expected to result in high genetic drift, and the increase in mutational load was determined. *S. typhimurium* accumulated mutations under these conditions such that after 1700 generations, 1% of the 444 lineages tested had suffered an obvious loss of fitness, as determined by decreased growth rate. These results suggest that in the absence of sex and with high genetic drift, genetic mechanisms, such as back or compensatory mutations, cannot compensate for the accumulation of deleterious mutations. In addition, we measured the appearance of auxotrophs, which allowed us to calculate an average spontaneous mutation rate of approximately 0.3–1.5 × 10^{-9} mutations per base pair per generation. This rate is measured for the largest genetic target studied so far, a collection of about 200 genes.**

Sex is genetically and ecologically an expensive and cumbersome means of reproduction, suggesting that it must provide some significant advantage(s) for those systems which utilize it (see, for example, refs. 1–3). One of several theories which have been proposed to explain the prevalence of sex is that sex may rearrange the genetic load in the population to continually generate some individuals with a fit combination of alleles. This theory is encapsulated in the hypothesis known as Muller's ratchet (4, 5). According to this hypothesis, the accumulation of deleterious mutations can lead to the loss of the most fit (mutation-free) class from a population. Muller (4) noted that this problem is most acute in asexual lines of descent. This problem will be exacerbated by high mutation rates and by small population sizes. Such lineages will accumulate deleterious mutations, and in a population of finite size, genetic drift will inevitably result in the irreversible loss of the least mutated class, unless back mutations occur at a high rate. As a result, the mutational load will increase in a rachet-like manner with the successive loss of the least mutated class. For any particular set of circumstances, the effects of Muller's ratchet can be slowed or stopped four different ways: (*i*) Increasing population size minimizes the effects of the ratchet by allowing selection to operate on the individuals that escape deleterious mutation, ensuring that the least mutated class is always present. (*ii*) Reducing the mutation rate decreases the accumulation of added load and makes it more likely that the least mutated class is represented. (*iii*) Increasing the frequency of compensating mutations makes it more likely that the accumulated mutations will not result in decreased fitness. (*iv*) Allowing sexual recombination permits rearrangements of deleterious mutations and increases the number of individuals that are free of deleterious mutations.

The application of the ratchet to real-life situations and the potential importance of sex in opposing it, have been experimentally examined in only three cases. (*i*) Bell (6) analyzed and reinterpreted previous data on "senescence" in protozoa and suggested that the loss of viability observed in isolated, asexually propagated protozoa lineages is the result of the ratchet. (*ii*) Chao (7) showed that the RNA virus ϕ6, when propagated asexually under intense genetic drift, accumulated deleterious mutations resulting in loss of viability. (*iii*) Similarly, others (8, 9) have shown that vesicular stomatitis virus (VSV) lost fitness when genetic bottlenecks were introduced during virus propagation. Chao *et al.* (10) showed that recombination between mutant ϕ6 lineages could restore viability almost to the wild-type level, demonstrating how sex can slow down the ratchet. However, the ϕ6 and VSV cases are exceptional in that these RNA viruses exhibit an extremely high mutation rate of 10^{-5} to 10^{-3} mutations per base pair per generation (11, 12). We were therefore interested in examining the significance of Muller's ratchet in a DNA-based genetic system with a more typical genomic mutation rate (13).

MATERIALS AND METHODS

Bacterial Strains, Media, and Growth Conditions. The bacterial strain *Salmonella typhimurium* LT2 was used for the experiment. Cells were propagated at 37°C by daily repeated single-colony streaking on LB (Luria–Bertani) agar plates supplemented with 0.2% glucose and 3 mM $CaCl_2$. Growth rates of final clones were determined in liquid cultures at 37°C in LB with the above supplements. Auxotrophies were determined by adding various nutrient supplements to M9 minimal glucose agar plates and scoring for growth after incubation overnight at 37°C.

RESULTS AND DISCUSSION

Experimental Set-Up. For our study, we choose the genetically well-defined eubacterium *S. typhimurium*. We performed the experiment under conditions in which sex by the bacteria was not allowed. A total of 444 separate lineages were initiated from one parent colony by streaking cells on nutrient-rich-agar plates. Each lineage was artificially forced through a bottleneck of one random single cell by picking cells from one colony and streaking for single colonies. The expansion of a single cell to a colony after an overnight incubation required approximately 28 generations of growth, which we define as one cycle of growth. This procedure is expected to provide an intense genetic drift since the population size is decreased to one random individual for each growth cycle. The 444 lineages were each run for an average of 60 growth cycles (≈1700 generations) each.

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Appearance of Slow Growers. To determine if the various lineages had accumulated mutations, we screened for mutants with altered growth rates by visually inspecting colony size (Table 1). At the end of the experiment, we found 5 strains among the 444 lineages that grew significantly slower than wild type (3 of these 5 were also auxotrophic), but, importantly, no mutants growing faster than the wild type were observed. Of the five slow-growers the generation time ranged from 25.0 to 47.5 min, as compared with 23.2 min for the wild-type parent. We can conclude that 5 of 444 lineages (1%) suffered a significant loss of fitness after 1700 generations. These results suggest that Muller's ratchet indeed operates at a significant rate in bacteria and that genetic mechanisms, such as back or compensatory mutations, cannot prevent the accumulation of deleterious mutations under the conditions used. If back or compensatory mutations are unable to halt the ratchet, how are its effects avoided in bacteria? As discussed previously, either a large population combined with selection, assuring the presence of a mutation-free wild-type individual at all times, or the occurrence of sex, allowing restoration of the wild-type genotype by recombination, could modulate the effects of the ratchet. The effective population size is not well known in bacteria, but if transmission to new hosts and environments frequently occurs by a single or a few individuals, then genetic drift would be high, and the effects of the ratchet would be intensified. Furthermore, even in the absence of transmission bottlenecks, bacterial populations could be subject to effective physiological bottlenecks due to temporal variations in their environments. Under either of these conditions, sex might be an important factor opposing the ratchet.

Calculation of the Spontaneous Mutation Rate. To measure the spontaneous mutation rate in this experiment, we scored the appearance of auxotrophic mutants. Since the experiments were conducted on nutrient-rich medium, auxotrophic mutations are assumed to be selectively neutral. At the end of the experiment we found 16 auxotrophs among the 444 lineages (Table 2). From these data, we can estimate an average genomic mutation rate which is based on the largest genetic target studied so far and which does not rely on the extrapolation of a mutation rate from one specific target gene or region—i.e., the *lacI* or *his* operon genes (13). In the *S. typhimurium* genome of 4800 kbp (14), ≈200 genes are involved in the biosynthetic pathways for amino acids, purines, pyrimidines, vitamins, etc. (15). In looking for auxotrophs, we can detect mutations in any of these 200 genes assuming that the mutation causes a sufficient loss of function. From the target size (about 200 kbp), the number of defined auxotrophs found (nine), the total number of generations passed (7.4×10^5), and the penetrance of the mutations (4–20%), we calculated a mutation rate of $\approx 0.3–1.5 \times 10^{-9}$ mutations per base pair per generation (0.0014–0.0072 mutations per genome per generation). The variables in this calculation relate to the nature of the spontaneous mutations causing auxotrophy and the penetrance of those mutations—i.e., the proportion of spontaneous mutations which result in loss of function and auxotrophy. We have assumed that most of the mutations are base-pair substitutions. This assumption is supported by the spectrum of *his* operon auxotrophs isolated in *S. typhimurium*, where 67% are base-pair substitutions (see ref. 13 and references therein). Regarding penetrance, the lower extreme of a 4% penetrance is based on the assumption that only nonsense mutations result in auxotrophy. The 20% value is derived from *lacI* and *his* operon mutation data, which show that among loss of function mutations missense mutations are three to four times as common as nonsense mutations (13, 16). The calculated rate is very similar to what has been determined previously from more limited targets. The *his* operon and *lacI* rates are 0.5×10^{-9} and $0.4–0.7 \times 10^{-9}$ mutations per base pair per generation, respectively (15), and our measurement thus provides an additional independent confirmation of these potentially biased numbers.

Table 1. Generation time for the five mutants found

wt	mut9	mut4	mut20	mut5	mut3
23.2 ± 0.7	25.0 ± 1.1	25.1 ± 1.0	27.0 ± 1.3	46.5 ± 1.1	47.5 ± 3.5

The generation time (in min) for each slow grower represents the average of at least six measurements. Values are mean ± the standard deviation within a 95% confidence limit. wt, Wild type.

Table 2. Types of auxotrophs found

Thi⁻ (3)	Ade⁻ (2)	Met⁻ (1)	Asp⁻ (1)	Trp⁻ (1)	Arg⁻ (1)	Other* (7)

The number of each mutant found is given in parentheses.
*Other refers to a complex/leaky phenotype.

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