

Long term evolution experiment



Brian Baer in Fox & Lenski, 2015

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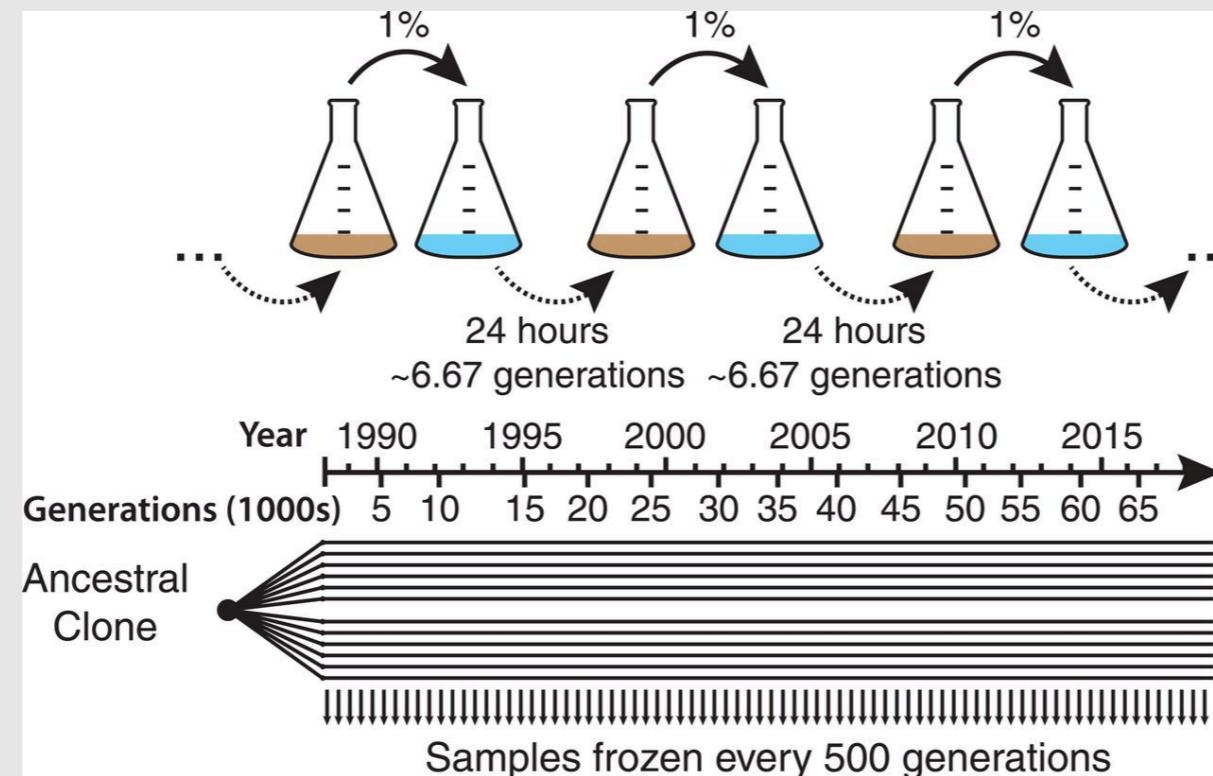
Learning objectives

- Make predictions of long term evolution
- Understand a key experiment
- Discuss repeatability of evolution

The baseline strain used in this study is an *Escherichia coli* B clone that has also been used in several other evolutionary studies (Chao et al. 1977; Lenski and Levin 1985; Bouma and Lenski 1988; Lenski 1988a, 1988b). *Escherichia coli* B has been used in the laboratory for many decades. During this time, it has undoubtedly been cultured and stored under a variety of conditions, some of which may have been similar to those employed in the experiments reported here. Ever since this strain has been used in evolutionary studies, it has been stored frozen as a clonal isolate, essentially without evolution. We view the culture conditions in the present study as a “novel” environment, although we recognize that our founding strain may have previously adapted to similar conditions. In any case, our results seem to exclude the possibility that the founding strain was already well adapted to the culture conditions used in our experiments. This strain carries no plasmids and harbors no functional bacteriophages; it is strictly asexual. It is also prototrophic but unable to grow on the sugar L-arabinose (Ara^-). It is resistant to coliphage T6 but sensitive to other T-phages, including T5.

The culture medium used in all experiments was Davis minimal broth (Carlton and Brown 1981) supplemented with 2×10^{-6} g thiamine hydrochloride and 0.025 g glucose per liter (Lenski 1988a), which permits a stationary phase bacterial density of about 5×10^7 cells per mL. Culture volume was 10 mL maintained in 50-mL Erlenmeyer flasks set in a shaking incubator at 37°C and 120 rpm. Cultures were propagated daily by transferring 0.1 mL of each culture into 9.9 mL of fresh medium. In the course of this 24-h cycle, bacterial populations attained stationary phase densities. The resulting 100-fold daily growth of each bacterial population represents ~ 6.64 generations of binary fission.

Lenski et al. 1991

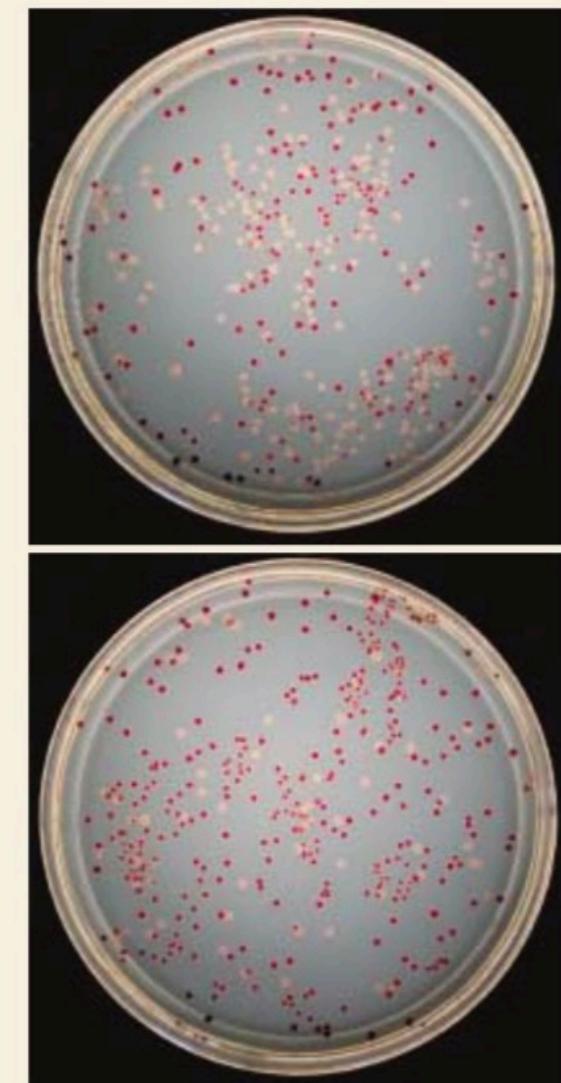
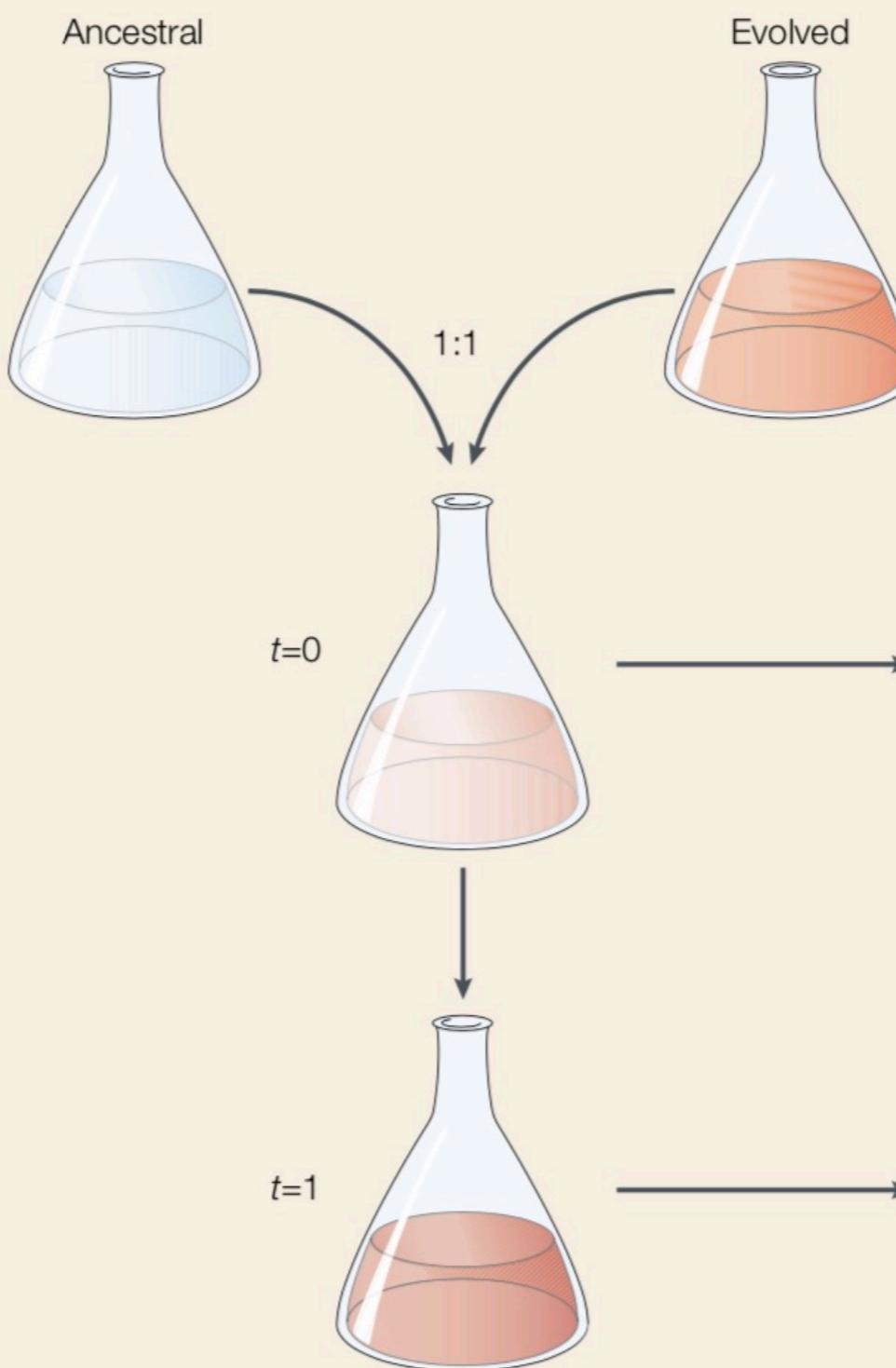


Blount et al. 2018

Box 2 | Measuring fitness

The fitness of an evolved type is generally expressed relative to its ancestor. Relative fitness is measured by allowing the ancestral and evolved types to compete with one another. Unless otherwise specified, the competition environment is the same as that used for the experimental evolution. The following description presents the protocol used in the long-term serial-transfer experiment with *Escherichia coli*^{13,25,26}, but similar procedures are used in experiments with many microorganisms.

The two competitors are grown separately in the competition environment to ensure that they are comparably acclimated to the test conditions. They are then mixed (usually at a 1:1 ratio) and diluted (100-fold in this case) in the competition environment. Initial densities at timepoint $t = 0$ are estimated by diluting and spreading the cells on an indicator agar that distinguishes the evolved and ancestral types by colony colour, which differs owing to an engineered marker that is selectively neutral. In this case, red and white colonies correspond to Ara⁻ and Ara⁺ phenotypes, respectively. After one day ($t = 1$) (corresponding to the serial-transfer cycle in the evolution experiment), final densities are estimated by plating cells, as before, on the indicator agar. The growth rate of each competitor is calculated as the natural logarithm of the ratio of its final density to its initial density (adjusted for dilution during plating). Relative fitness is then defined simply as the ratio of the realized growth rates of the evolved and ancestral types.



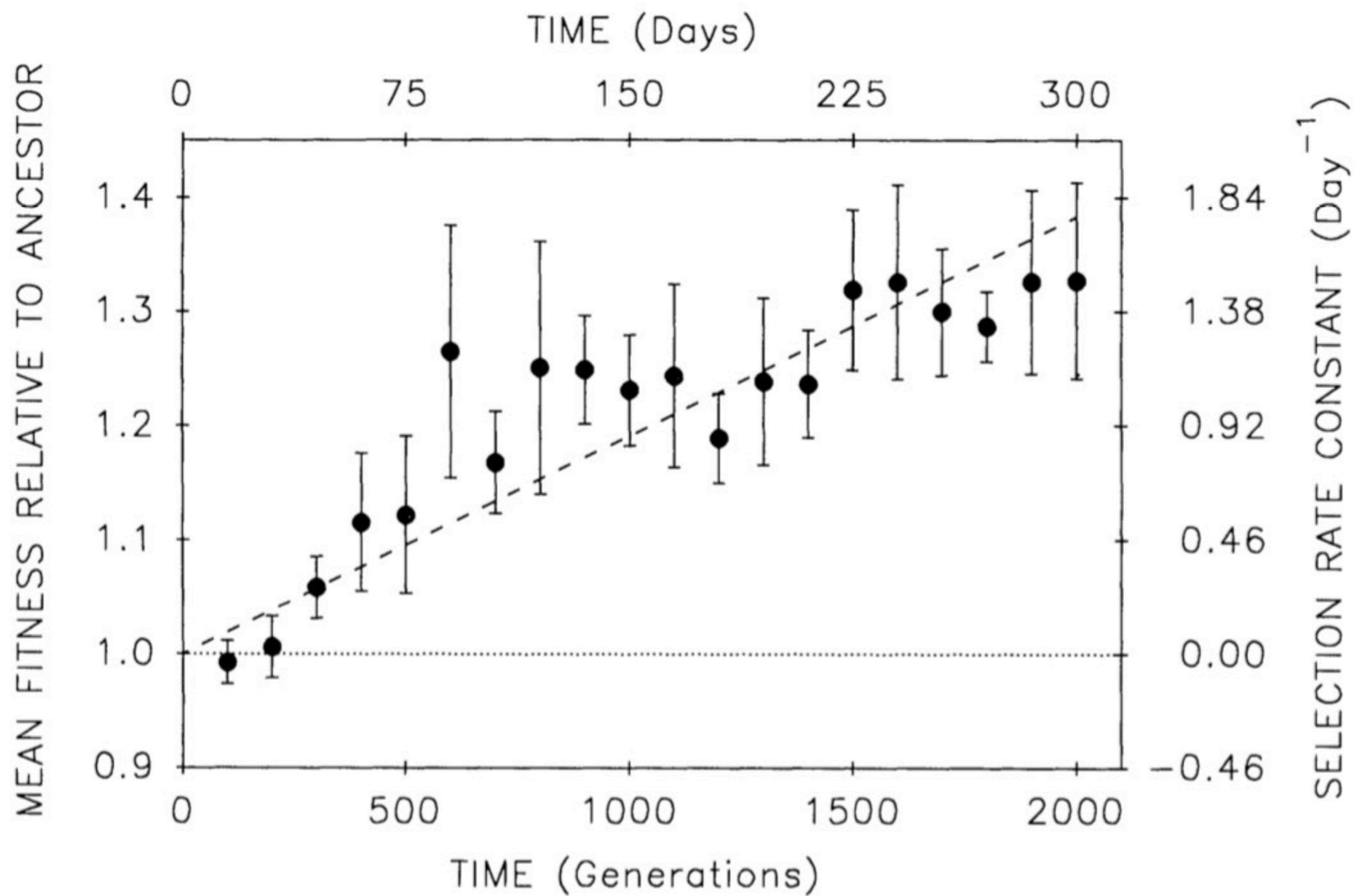


FIG. 1.—Trajectory of mean fitness during 2,000 generations (300 d). Fitness is expressed relative to an ancestral strain that has a neutral genetic marker; the selection-rate constant is related to fitness as described by eqq. (2b) and (3c). Mean fitness was estimated for each of the 12 evolving populations at 100-generation intervals. *Filled circles*, grand means of 12 estimates of mean fitness. The error bars show the 95% confidence interval based on the t distribution with 11 df. *Dashed line*, least squares linear regression of mean fitness against time, with the intercept constrained to 1. The slope of this line was calculated as the mean of the 12 slopes obtained from separate regressions for each of the 12 populations (table 1).

Throughout the duration of the LTEE, there has existed an ecological opportunity in the form of an abundant, but unused, resource. DM25 medium contains not only glucose, but also citrate at a high concentration. The inability to use citrate as an energy source under oxic conditions has long been a defining characteristic of *E. coli* as a species (35, 36). Nevertheless, *E. coli* is not wholly indifferent to citrate. It uses a ferric dicitrate transport system for iron acquisition, although citrate does not enter the cell in this process (37, 38). It also has a complete tricarboxylic acid cycle, and can thus metabolize citrate internally during aerobic growth on other substrates (39). *E. coli* is able to ferment citrate under anoxic conditions if a cosubstrate is available for reducing power (40). The only known barrier to aerobic growth on citrate is its inability to transport citrate under oxic conditions (41–43). Indeed, atypical *E. coli* that grow aerobically on citrate (Cit^+) have been isolated from agricultural and clinical settings, and were found to harbor plasmids, presumably acquired from other species, that encode citrate transporters (44, 45).

Despite this potential, none of the 12 LTEE populations evolved the capacity to use the citrate that was present in their environment for over 30,000 generations. During that time, each population experienced billions of mutations (22), far more than the number of possible point mutations in the \approx 4.6-million-bp genome. This ratio implies, to a first approximation, that each population tried every typical one-step mutation many times. It must be difficult, therefore, to evolve the Cit⁺ phenotype, despite the ecological opportunity. Here we report that a Cit⁺ variant finally evolved in one population by 31,500 generations, and its descendants later rose to numerical dominance. The new Cit⁺ function has been the most profound adaptation observed during the LTEE and has had major consequences. As we will show, the population achieved a severalfold increase in size. Moreover, a stable polymorphism emerged, with a Cit⁻ minority coexisting with the new Cit⁺ majority. Interestingly, the population that evolved the Cit⁺ function is not one that had previously become hypermutable. It is also intriguing that this key innovation evolved so late in the experiment, given that the rate of fitness improvement had declined substantially in all of the populations (3, 23).

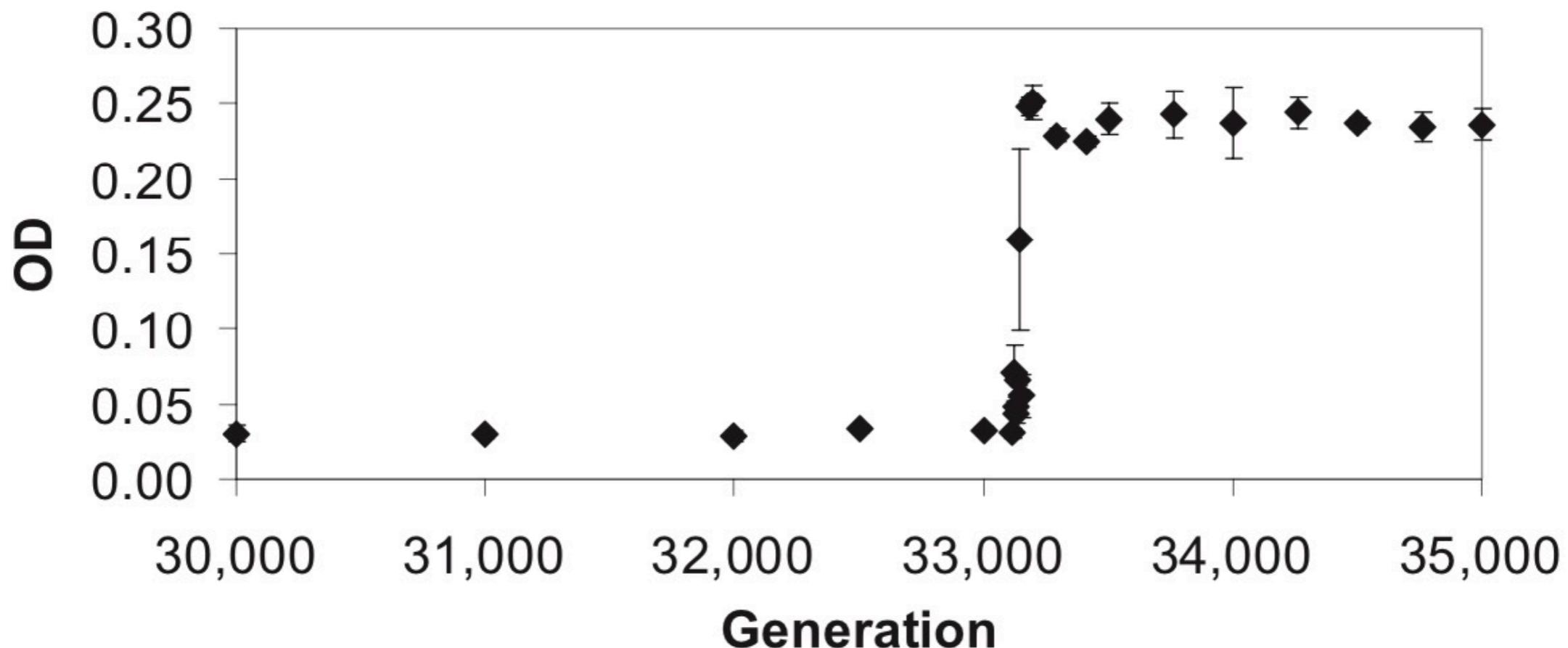


Fig. 1. Population expansion during evolution of the Cit⁺ phenotype. Samples frozen at various times in the history of population Ara-3 were revived, and three DM25 cultures were established for each generation. Optical density (OD) at 420 nm was measured for each culture at 24 h. Error bars show the range of three values measured for each generation.

Table 1. Summary of replay experiments

Generation	First experiment		Second experiment		Third experiment	
	Replicates	Independent Cit ⁺ mutants	Replicates	Independent Cit ⁺ mutants	Replicates	Independent Cit ⁺ mutants
Ancestor	6	0	10	0	200	0
5,000	—	—	—	—	200	0
10,000	6	0	30	0	200	0
15,000	—	—	—	—	200	0
20,000	6	0	30	0	200	2
25,000	6	0	30	0	200	0
27,000	—	—	—	—	200	2
27,500	6	0	30	0	—	—
28,000	—	—	—	—	200	0
29,000	6	0	30	0	200	0
30,000	6	0	30	0	200	0
30,500	6	1	30	0	—	—
31,000	6	0	30	0	200	1
31,500	6	1	30	0	200	1
32,000	6	0	30	4	200	2
32,500	6	2	30	1	200	0
Totals	72	4	340	5	2,800	8

“The first set of questions, about the dynamics of adaptation, had clear expectations that were testable in a fairly standard hypothesis-driven framework. For example, I was pretty sure we would see the rate of fitness improvement decelerate over time, and it has; and I was also pretty sure we’d see a quasi-step-like dynamic to the early fitness increases, and we did. Nonetheless, these analyses have yielded surprises as well, including evidence that fitness can increase indefinitely, and essentially without limit, even in a constant environment. In regard to the second set of questions, about the dynamics of genome evolution and their coupling to phenotypic changes—I’m sure these were part of my original thinking, but I admit that I had almost no idea how I would answer them. Hope sprung eternal, I guess; fortunately, wonderful collaborators, like the molecular microbiologist Dom Schneider, and new technologies—wow, sequencing entire genomes—saved the LTEE.”