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STEPS: Serially Transferred Evolving Population Simulator

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Software

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Summary

Bacteria and other microbes are widely used in the field of experimental evolution (Ascensao & Desai, 2025; Lenski, 2017; McDonald, 2019). In many of these experiments, the populations are propagated by periodic serial dilutions into fresh media, with the microbes growing to a maximum population size between successive transfers. Here we present the Serially Transferred Evolving Population Simulator (STEPS) software, which simulates the dynamics of asexual populations as they grow and evolve in these experiments. The STEPS software allows new mutations to occur while a population grows, including beneficial, neutral, and deleterious mutations. The resulting lineages then grow at different rates according to the fitness effects of their accumulated mutations. After the total population reaches the maximum size, the dilution factor determines the proportion of individuals that are randomly chosen to start the next growth cycle. The differential growth rates of the lineages reflect the process of natural selection, while the periodic bottlenecks impose random genetic drift.

Statement of need

The Long-Term Evolution Experiment (LTEE) with *Escherichia coli* was started in 1988 (Lenski et al., 1991; Lenski, 2023) and it continues to this day (Barrick et al., 2023). The LTEE has been used to study many aspects of evolutionary dynamics (Blount et al., 2008; Couce et al., 2024; Tenaillon et al., 2016; Wiser et al., 2013), and it has inspired many other experiments that use similar approaches (Izutsu et al., 2024; Johnson et al., 2021; Stroud & Ratcliff, 2025). Various publications based on these projects have included simulations to analyze and interpret the experimental results. The authors of these papers have written new simulation software that is custom-built to a specific experimental system or result, and even publications based on the same system may use different simulation methods (e.g., Good et al., 2017; Wiser et al., 2013). We anticipate that future studies will be able to use STEPS to perform relevant quantitative analyses or interpret qualitative results. Researchers may also use STEPS to design new experiments or as a framework on which to build customized simulations. We expect that employing the STEPS program in these various ways will improve consistency and efficiency across studies.

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Software Design

To enable STEPS to be accessible to as many researchers as possible, we have produced two different ways to run the simulations. The first approach requires using a command-line interface to run STEPS, and it is intended for advanced applications, such as generating data for large numbers of replicate populations for statistical analysis and comparison to experimental results. The second approach uses a web-based graphical user interface that provides immediate visualization of the results, helping researchers develop their intuition about the dynamics of evolving populations under various scenarios. This version of STEPS can also be used in educational settings, allowing students without computational backgrounds to obtain results quickly and easily. The web-based version was inspired, in part, by the Avida-ED software used to promote a better understanding of evolution through experiments with agent-based digital organisms in a virtual world (Smith et al., 2016). Some features of the command-line version of STEPS are not available in the web-based version. However, both versions use the same underlying code while the simulations are running.

The underlying mechanics of the STEPS program are designed to simulate the biological processes that occur in actual evolution experiments with microorganisms, while taking advantage of computational methods that allow the simulations to proceed quickly and efficiently. For example, the timesteps in the simulations correspond approximately to the population's doubling time, providing meaningful timepoints for introducing mutations. The mutational categories, the effects of mutations on growth rates, and the serial transfer process have been implemented such that the dynamics of the simulated populations reflect the same interplay of mutation, selection, and drift that occurs in biological experiments. The underlying theoretical framework for STEPS was inspired, in part, by simulations published previously (Good et al., 2017; Wiser et al., 2013). However, STEPS has been designed and written to allow for easier access, wider use, and future development.

Research impact statement

The first version of STEPS was developed for and used extensively in a peer-reviewed paper on the effects of population bottlenecks on the dynamics of experimentally evolving bacteria (Izutsu et al., 2024). Since then, the STEPS program has undergone significant development including (i) refinements to speed up the simulations, (ii) creation of a web-based version to complement the command-line version, and (iii) writing an in-depth user manual to help both beginning and advanced users better understand both the STEPS software and the population dynamical processes that it simulates. The user manual is freely available (Lake et al., 2025) and has been downloaded more than 250 times since it was posted (August 1, 2025).

With the rapid growth of both experimental evolution and microbial genomics, we expect many research groups will find similar utility in the STEPS program, and we look forward to its further development by ourselves and others for future needs that may arise. Indeed, we are using STEPS in our ongoing research. For example, it is difficult to estimate the fraction of observed mutations that are beneficial drivers versus neutral hitchhikers using empirical data alone. However, one can estimate that fraction by comparing empirical data with STEPS simulations that include only beneficial mutations with alternative distributions that also include neutral and even deleterious mutations.

We also expect the STEPS program to be used for teaching core principles of evolution, in general, and microbial evolution, in particular, in both undergraduate and graduate courses, including those that have a laboratory-based component.

Key features

The figures below illustrate a few of the dynamical outputs that are available with the STEPS package. Figure 1 shows the trajectories of (A) average fitness and (B) average accumulated mutations for 12 simulated populations over the course of 2,000 generations. The essential biological parameter values are listed in the figure legend; they correspond to the approximate values for the LTEE. Both trajectories exhibit steplike dynamics that are typical of large microbial populations in which *de novo* mutations drive adaptation (Lenski & Travisano, 1994), and which inspired the name of the STEPS program.

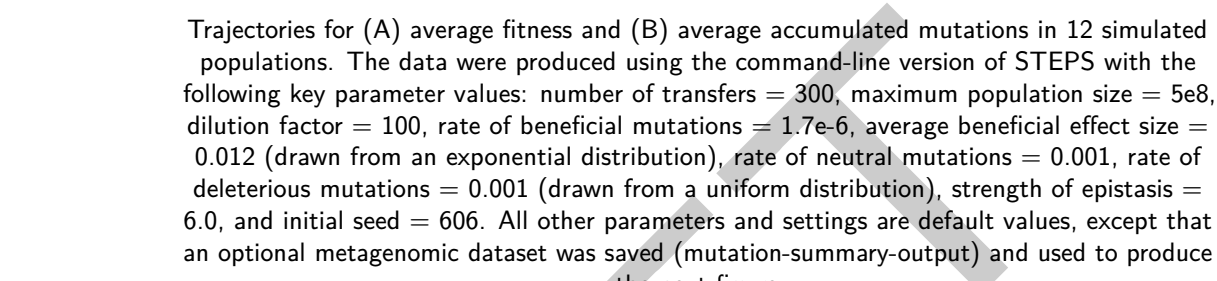

Figure 1: Trajectories for (A) average fitness and (B) average accumulated mutations in 12 simulated populations. The data were produced using the command-line version of STEPS with the following key parameter values: number of transfers = 300, maximum population size = 5e8, dilution factor = 100, rate of beneficial mutations = 1.7e-6, average beneficial effect size = 0.012 (drawn from an exponential distribution), rate of neutral mutations = 0.001, rate of deleterious mutations = 0.001 (drawn from a uniform distribution), strength of epistasis = 6.0, and initial seed = 606. All other parameters and settings are default values, except that an optional metagenomic dataset was saved (mutation-summary-output) and used to produce the next figure.

Figure 2 plots the metagenomic data for one of the 12 populations from the optional file that recorded the trajectories of every mutation that reached a threshold frequency. One can see a clean selective sweep in the first few hundred generations, followed by more complex dynamics as beneficial mutations (alone and in combination) compete with one another. This competition leads to the phenomenon of clonal interference, whereby many beneficial mutations are outcompeted by lineages that acquire other mutations that confer even larger benefits (Gerrish & Lenski, 1998; Levy et al., 2015).

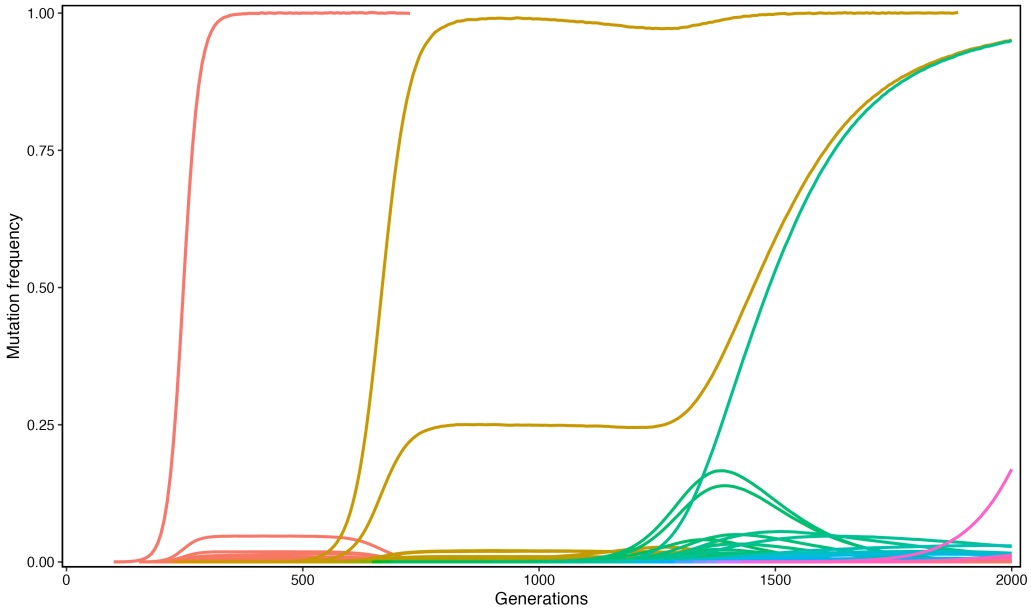

Figure 2: Trajectories for the frequencies of individual mutations in one of the populations in Figure 1 (brown trajectory, third population from left to show a conspicuous increase in fitness.) Only those mutations that reached a frequency of at least 0.01% were saved in the output file. See Figure 1 for parameter values and other details.

To facilitate the use of STEPS by users with diverse interests and skillsets in both research and educational settings, we have produced a User Manual (Lake et al., 2025) that explains: the scientific context and purpose of the STEPS software (Chapter 1); the use of the web-based version, including numerous exercises with figures (Chapter 2); the setup and full set of options available in the command-line version (Chapter 3); and the mechanics of the simulations that underpin both the command-line and web-based versions (Chapter 4). Figure 3 is a screenshot of the web interface along with results generated in a matter of seconds using the default settings.

Figure 3: Screenshot of the interface and results using the web-based version of STEPS. Default parameter values were used, except that the random seed was set to 606. These values are the same as used for Figure 1, except that the default values for the neutral and deleterious mutation rates are set to 0 to allow faster runtimes. Exercises presented in the User Manual (Lake et al., 2025) explore the effects of adding these types of mutations, varying population sizes, extending run durations, changing other parameters, and using additional options.

AI usage disclosure

No generative AI tools were used in the development of this software, the writing of this manuscript, or the preparation of the supplemental STEPS User Manual.

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