



Best Practices in Microbial Experimental Evolution

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Received: 3 May 2023 / Accepted: 11 May 2023 / Published online: 20 May 2023

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Scientific disciplines are dynamic: they grow and evolve, they fuse with one another and divide. As fields expand and contract and their boundaries are often ill-defined and overlapping. For any field, no definition will satisfy all of its practitioners. In the early days of molecular biology there was debate over the scope of this new field and criticism of the name “molecular biology” itself (Waddington 1961; Astbury 1961). Crick sidestepped this whole issue by asserting that “molecular biology can be defined as anything that interests molecular biologists” (Crick 1970).

Like molecular biology in the mid-twentieth century, the field of experimental evolution has grown steadily over the span of several decades, from a niche field dominated by a small number of groups to a robust field attracting an influx of new talent and new ideas. The strength of experimental evolution (and one of the reasons for the growth of the field) is the ability to perform experiments that are impossible or impractical in natural populations and to quantify fundamental parameters that drive evolutionary processes. At the same time, experimental evolution is a powerful tool for understanding the biology of the organisms we employ. By identifying the genes and pathways that respond to selection, experimental evolution is a powerful tool for assigning functions to genes and revealing previously unknown connections between cellular processes.

In the last several decades, the number of articles published each year with the keyword “experimental evolution” have increased over ten-fold (Fig. 1). As scientific disciplines grow, they develop their own culture, methods, and tricks-of-the-trade. Experimental evolution is no exception. The goal of this Special Issue of the *Journal of Molecular Evolution* is to begin collecting these tips and tricks in one

place. With thousands of new articles each year on experimental evolution, we chose to focus this issue on laboratory evolution experiments using either bacteria or fungi. In this issue, therefore, we do not cover the excellent laboratory evolution experiments in metazoans (Rose 1984), which pre-date—and motivated—the Lenski long-term experiment with *E. coli* (see (Lenski 2023)). Nor do we attempt to include best practices for field experiments on evolution (e.g., (Barrett et al. 2019)) or the evolution of phages or viruses (Bull et al. 1997; Wichman and Brown 2010). For broader reviews of experimental evolution including these fields see (Garland and Rose 2009; Burke and Rose 2009; Kawecki et al. 2012; Cooper 2018).

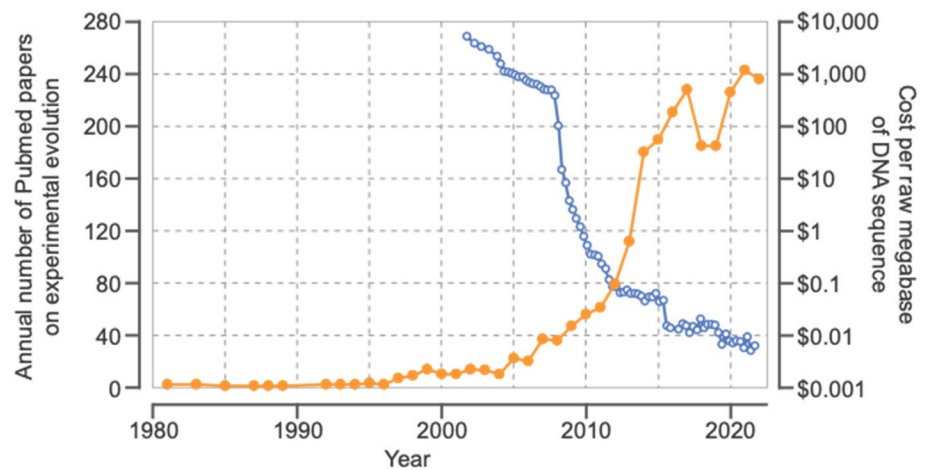
In this issue, authors present their latest best practices, tips and tricks to improve the precision, quality, reproducibility, and impact of laboratory evolution experiments in microbes. The articles in this issue, therefore, go beyond what is typically reported in the methods sections of published papers: they are part methods, part results, and part commentary. Broadly, the papers in this issue can be viewed on three axes. First—since the features of the organism will necessarily impact experimental design—is whether the study system is yeast (Burke 2023; Smukowski Heil 2023; Martínez and Lang 2023; Spealman et al. 2023; Kinsler et al. 2023) or bacteria (Theodosiou et al. 2022; Worthan et al. 2023; Lenski 2023; Limdi et al. 2023). Second is whether the experiments are of the “classic” type relying on long-term propagation followed by whole-genome sequencing and comparison across replicate experiments to tease apart which mutations are adaptive (Burke 2023; Smukowski Heil 2023; Worthan et al. 2023; Lenski 2023; Martínez and Lang 2023). Or, alternatively, whether the experiments proceed for only a short time because they leverage DNA barcodes to track thousands of clonal replicate strains as they each accumulate usually a single adaptive mutation (Theodosiou et al. 2022; Johnson et al. 2023; Li et al. 2023; Kinsler et al. 2023; Limdi et al. 2023). A third way to separate the articles, and the way in which we’ve organized this special issue (see Table of Contents), is by topic, whether they focus principally on initial setup (Theodosiou et al. 2022; Johnson et al.

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Fig. 1 Growth of the field of Experimental Evolution. The annual number of pubmed-accessible publications using the keyword “experimental evolution” has risen steadily over the last several decades. The sharp rise in publications coincides with a drop in the cost of DNA sequencing. DNA sequencing cost data is from NHGRI (www.genome.gov)



2023; Burke 2023; Lenski 2023), on quantifying phenotypes and fitness of the evolved strains (Worthan et al. 2023; Li et al. 2023; Limdi et al. 2023; Kinsler et al. 2023), or on identifying causative mutations underlying changes in phenotypes (Smukowski Heil 2023; Martínez and Lang 2023; Spealman et al. 2023).

Best Practices for Setting up an Evolution Experiment

Evolutionary biologists are enamored by the concept of historical contingency, where chance events (or arbitrary choices between ostensibly equivalent options) can have a profound impact on the future (Gould 1989; Blount et al. 2018). Most experimentalists have encountered a time when, at the completion of an experiment, they look back wishing that they had had the foresight to set things up differently: If only I had used a different strain background. If only I had included this other control. For laboratory evolution experiments that may well run for years—or even decades—choices made at the outset can affect an investigator’s research well into the future. In the first article of this section, Richard Lenski looks back in time to revisit the initial setup of his foundational long-term evolution experiment in *E. coli* (Lenski 2023). Specifically, this article discusses the choices that were made regarding strain background, growth conditions, medium, and propagation regime—choices that led to the success of the longest-running laboratory evolution experiment. The next article in this section discusses challenges involved in setting up a very high-replicate type of evolution experiment that leverages DNA barcodes (Levy et al. 2015) in an organism where this type of experiment has not been done before (Theodosiou et al. 2022). These barcodes label otherwise identical genotypes such that researchers can study many possible first-step mutations that could contribute to

adaptation. This article is followed by another discussing best practices for designing and identifying DNA barcodes in a way that improves ability to track evolutionary dynamics (Johnson et al. 2023). The final article in this section discusses how to expand the complexity of evolution experiments beyond single genotypes to include genetically diverse starting populations (Burke 2023).

Best Practices for Measuring Fitness of Evolved Strains

Several challenges arise when isolating evolved strains and measuring the changes in their fitness relative to the ancestor of an evolution experiment. One challenge is that these experiments often seek to recapitulate the conditions of the original evolution experiment. The first two articles in this section report that this can be tricky business, as fitness measurements are extremely sensitive to subtle differences from one experiment to the next (Worthan et al. 2023; Kinsler et al. 2023). The next article in this section discusses how to design fitness measurements that quantify any deleterious effects that evolved mutants may have (Limdi et al. 2023). Though evolved mutants are typically adaptive in the original evolution condition, they often come with disadvantages in other environments, and characterizing the frequency of these tradeoffs is a major goal within the field of experimental evolution (Herren and Baym 2022; Leiby and Marx 2014; Bono et al. 2017; Kinsler et al. 2020; Bakerlee et al. 2021). The last article in this section seeks more broadly to quantify the fitness of large collections of evolved strains, specifically when those strains are tracked using DNA barcodes (Li et al. 2023). This article presents improved software for inferring relative fitness from these type of data, a key improvement being that the software is now implemented in a more accessible platform (python).

Best Practices for Identifying Adaptive Mutations

Identifying the mutations that underlie evolution and give any evolved strains a fitness advantage can be challenging for a variety of reasons, including that neutral mutations may also be present, and that difficult-to-sequence mutations may underlie changes in fitness. The first article in this section reviews how to use parallel evolution experiments to disentangle adaptive mutations from the numerous passenger mutations that exist in the genomes of evolved strains (Martínez and Lang 2023). The next article discusses the challenge of identifying adaptive mutations when they are not single nucleotide changes but instead are copy number variants (Spealman et al. 2023). And the final article discusses another type of adaptive change that can be difficult to detect via traditional approaches, loss of heterozygosity (Smukowski Heil 2023).

Conclusion

A defining feature of the experimental evolution field is its reliance on reproducibility. We leverage replicate experiments to understand which aspects of the evolutionary process are repeatable and predictable (Lenski 2023), which strains have indeed gained fitness advantages (Kinsler et al. 2023), and which mutations are common targets of adaptation (Martínez and Lang 2023). New tools are emerging to improve reproducibility, some of which enable a large number of strains to be evolved and studied in replicate (Levy et al. 2015; Theodosiou et al. 2022; Johnson et al. 2023), and others of which improve the ways in which high-replicate data is analyzed (Li et al. 2023). But at its core, our field relies on strong communication between researchers to help one another design, build upon and reproduce previous experiments. Another defining feature in the field of experimental evolution is thus the strength of our community and our culture of openly sharing experimental methods and tips. This special issue is presented to continue bringing our community together, to inspire discussion of best practices, and to encourage continued collaboration and support among the communities' members.

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