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Strengths and weaknesses of experimental evolution

Sara Magalhães and Margarida Matos

Centro de Biologia Ambiental, Faculdade de Ciências da Universidade de Lisboa, Edificio C2,3^{0} Piso, Campo Grande, 1749-016, Lisbon, Portugal

A recent article in *TREE* [1] presented a heavily documented review of experimental evolution, addressing three major issues: main questions and applications; experimental design and study systems; and caveats and limitations. Here, we comment on the latter issue.

The soundness of a scientific method depends on not only its adequacy relative to the specific goals, but also the relative suitability of alternative methods. Without intending to be exhaustive, we focus on other methods that are frequently used in evolutionary biology, and compare them with, and contrast them to, experimental evolution. We briefly analyze the objectives and relative limitations of each, as we feel that this analysis was lacking in the original article [1].

The most widely used method in evolutionary biology, ever since Darwin, is the comparative method. By comparing traits between extant populations, the general goal of this method is to infer the evolutionary history underlying present diversity. Hence, it is widely used to trace phylogenies, for which it is the only methodology available. It also serves to infer the microevolutionary processes that have shaped current natural populations, based on the patterns observed. However, knowledge of the ancestral state of populations, required to infer such processes, is usually missing. Hence, this method often uses present populations as surrogates of the ancestral state of others. However, this relies on several assumptions, such as simple evolutionary history, repeatability of evolution, and so on [2]. Experimental evolution is free of such assumptions, as it is evolutionary biology in its most empirical sense, enabling researchers to follow microevolutionary processes directly and to establish a causal link between patterns and processes. Our own work helps illustrate how experimental evolution can unravel the pitfalls of a comparative approach. Magalhães *et al.* [3] showed that a genetic tradeoff may be incorrectly inferred by direct comparison of the performance of populations of spider mites adapting to different hosts, whereas the experimental evolution analysis, taking into account the common ancestral state, showed a positive covariance. Matos and collaborators showed that laboratory evolution in *Drosophila subobscura* varies across foundations, particularly for life-history traits that are less relevant to fitness [4]. The direct evolutionary trajectories observed differ from those inferred using different populations as 'surrogates' for evolutionary states across generations [5,6].

Another goal in evolutionary biology is to characterize the evolutionary potential of populations. One frequent approach is to use inbred lines in sexual populations, assuming that the range of values across lines is a good representation of the standing genetic variation of outbred populations. However, inbreeding artifacts may lead to overwhelmingly positive genetic correlations between life-history traits that are absent in outbred populations. The study of mutants and how they differ from the 'wild state' is another approach used. However, mutants often have low fitness, and selection acts against them, in practice suppressing their role in evolution. The study of the evolution of aging provides a good illustration of the contrasting outcomes of using such approaches. Disparities were reported using inbred lines, mutants, and experimental evolution, with only the latter providing evidence for Hamilton's analysis [7].

Characterizing the evolutionary potential of a population is also done using additive genetic variance–covariance (G) matrices between fitness-related traits. This tool may accurately predict the short-term evolution of a population. However, there is a long-standing debate on the stability of G-matrices, which is required for longer-term predictions [8]. Experimental evolution is a fundamental, complementary tool, as it can test the stability of G-matrices by following the evolutionary trajectory of populations over a longer period of time. Unfortunately, to our knowledge, no study has yet provided robust conclusions on this matter.

Recently, genome scans have been used to compare populations exposed to different selection pressures, as well as to characterize their evolutionary potential. Such scans are valuable, especially when combined with experimental evolution, thus enabling researchers to trace the genome-wide real-time evolution of replicated populations [9–11].

Finally, modeling is a heuristic tool in evolutionary biology, particularly for exploring complex scenarios, such as evolution in heterogeneous environments. Again, experimental evolution may be an added value, by providing empirical data to test and adjust such models [12].

Experimental evolution does have its own limitations. However, it is still one of the most powerful tools in evolutionary biology, especially when combined with other approaches. Its most important goal is to establish links between microevolutionary processes and patterns. This in turn helps disentangling the evolutionary and genetic mechanisms underlying adaptation and diversity, the main goal of evolutionary biology ever since Darwin.

Corresponding author: Magalhães, S. (nmagalhaes@fc.ul.pt)

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The value of complementary approaches in evolutionary research: reply to Magalhães and Matos

Tadeusz J. Kawecki[1], Richard E. Lenski[2], Dieter Ebert[3], Brian Hollis[1], Isabelle Olivieri[4] and Michael C. Whitlock[5]

[1] Department of Ecology and Evolution, University of Lausanne, CH 1717 Lausanne, Switzerland
[2] BEACON Center for the Study of Evolution in Action, Michigan State University, East Lansing, MI 48824, USA
[3] University of Basel, Zoological Institute, Vesalgasse 1, 4051 Basel, Switzerland
[4] Université Montpelier 2, CNRS, Institut des Sciences de l'Evolution, UMR 5554, 34095 Montpelier cedex 05, France
[5] Department of Zoology, University of British Columbia, Vancouver, V6T 1Z4, Canada

In their Letter to *TREE* [1], Magalhães and Matos criticize our review of experimental evolution [2] for not discussing the limitations of other research approaches used in evolutionary biology. Although we agree that the strengths of experimental evolution result in part from the ability to circumvent some of those limitations, we felt that discussing the limitations of comparative, phylogenetic, paleontological, and other approaches was beyond the scope of our paper. The power of experimental evolution is manifest in the breadth and depth of insights gained through its application, as we reviewed [2]. Nonetheless, experimental evolution does have its own particular limitations as a research approach, and it is important that practitioners of experimental evolution are aware of them to avoid incorrect interpretation of results.

We believe that the 'gold standard' for addressing many evolutionary questions is one that uses multiple research approaches and methods that address complementary aspects of the issues at hand. Theory provides a broad framework about what might be possible and links specific assumptions with predictions, thus inspiring empirical tests and sometimes generating unexpected new insights. Experimental evolution can show what outcomes are plausible and accessible in a biological system with particular properties (e.g., population size and mode of reproduction). Studies of phenotypic and genetic variation in natural populations can establish how much, and what kinds of, variation exist for traits of interest; such studies may also show whether a particular process has occurred in nature (e.g., by detecting signatures of recent selection in the genome). Molecular biology may reveal the mechanisms by which genetic differences give rise to variation in phenotypes and fitness. Finally, phylogenetically based comparative approaches (and, for traits preserved in the fossil record, paleontology) reveal which of many possible evolutionary scenarios actually occurred, and whether the processes or factors of interest are sufficiently important or general to contribute to broad-scale patterns of differentiation within, and among, species or higher taxa.

The concerted application of such complementary approaches can synergistically advance understanding of an evolutionary phenomenon. A case in point is the evolution of aging. Insights from Peter B. Medawar and George C. Williams, later formalized in mathematical models (e.g., [3]), posited that senescence is an expected outcome of evolution (rather than an unavoidable result of attrition) and, moreover, predicted testable connections between aging, reproduction, and extrinsic mortality. Experimental evolution studies verified those predictions under laboratory conditions and demonstrated that lifespan can evolve rapidly in either direction (e.g., [4]). Quantitative genetic studies of natural populations confirmed the existence of ample genetic variation in the rate of aging as well as antagonistic pleiotropy between early- and late-life fitness components (e.g., [5]), including in humans (e.g., [6]). Genomic methods are beginning to identify some of the underlying polymorphisms (e.g., [7]), while other high-throughput methods are shedding light on the architecture of lifespan and related traits (e.g., [8]). In addition, experi-

Corresponding author: Kawecki, T.J. (tadeusz.kawecki@unil.ch)

ments with mutants, transgenics, and pharmacological manipulations suggest candidate physiological mechanisms (e.g., insulin signaling) that might modulate the trade-off between early reproduction and lifespan, while comparative studies show that these mechanisms are highly conserved across metazoans [9]. Some candidate genes show predicted patterns of geographic variation in allele frequencies across populations (e.g., [10]). Finally, phylogenetically based comparative studies (e.g., [11]) indicate that the factors identified by theory and in evolution experiments drive much of the variation in lifespan across taxa. Although many questions remain, the application of complementary approaches has allowed the evolution of aging to become a mature area of research with some potentially important biomedical applications (e.g., [12]).

In conclusion, experimental evolution is a powerful approach for studying evolution based on its particular strengths [1,2] as well as its ability to complement other approaches. The growth of experimental evolution in recent years suggests that this approach was underutilized in the past. We hope and expect that evolutionary biologists will continue to use all available approaches, alone and in concert, to advance understanding of evolution.

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Transcriptomics and microbial eukaryote diversity: a way forward

Shauna A. Murray[1,2], David J. Patterson[3,*] and Anne E. Thessen[3]

[1] School of Biotechnology and Biomolecular Sciences, University of New South Wales, Kensington, Sydney, NSW 2052, Australia
[2] Sydney Institute of Marine Sciences, Chowder Bay Rd, Mosman, NSW 2088, Australia
[3] Center for Library and Informatics, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

Recent reviews have pointed out the large proportion of microbial eukaryotic (protist) diversity that has yet to be described, and the enormous challenges that accompany its description [1]. The ~100 000 species [2] of microbial eukaryotes may be significantly underestimated if we continue to discover increasing numbers of cryptic species. Tools such as environmental sequencing, which have been highly successful in revealing novel lineages of uncultured bacteria and archaea, promise to add substantial new data for comparatively little effort [1]. In our opinion, significant challenges, grounded in both biology and the history of study of these organisms, need to be faced before environmental sequencing can unlock our understanding of microbial eukaryotic diversity.

First, species concepts for eukaryotes differ from those applied to prokaryotes. In bacteria and archaea, commonly used molecular sequencing techniques are now an integral part of species concepts [3], with terminal taxa (species) often distinguished by a percentage difference in a marker gene, typically 16S rDNA. Species concepts of eukaryotes tend to be more theoretically based. Typological or biological species concepts are common approaches: morphological differences and sometimes reproductive isolation are examined. That is, the framework for our understanding of species of microbial eukaryotes is not solely or primarily based on molecular sequence differences. Biological differences tend to support the existence of a clearer species boundary in eukaryotes: complex life cycles involving sexual stages may occur, higher barriers to lateral gene transfer are usually present, and significant population-level diversity often occurs [4], indicating that speciation could be investigated using coalescent theory. In contrast, bacteria and archaea have simpler inheritance mechanisms interwoven with large-scale lateral gene transfer, causing the 'edges' of species to be particularly 'fuzzy' [5]. In this context, the application of a genetic distance measure as a marker appears a highly practical, and at times, the

Corresponding author: Murray, S.A. (s.murray@unsw.edu.au)
* Current address: School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA.