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Review

Experimental evolution in fungi: An untapped resource ☆

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ABSTRACT

Historically, evolutionary biology has been considered an observational science. Examining populations and inferring evolutionary histories mold evolutionary theories. In contrast, laboratory evolution experiments make use of the amenability of traditional model organisms to study fundamental processes underlying evolution in real time in simple, but well-controlled, environments. With advances in high-throughput biology and next generation sequencing, it is now possible to propagate hundreds of parallel populations over thousands of generations and to quantify precisely the frequencies of various mutations over time. Experimental evolution combines the ability to simultaneously monitor replicate populations with the power to vary individual parameters to test specific evolutionary hypotheses, something that is impractical or infeasible in natural populations. Many labs are now conducting laboratory evolution experiments in nearly all model systems including viruses, bacteria, yeast, nematodes, and fruit flies. Among these systems, fungi occupy a unique niche: with a short generation time, small compact genomes, and sexual cycles, fungi are a particularly valuable and largely untapped resource for propelling future growth in the field of experimental evolution. Here, we describe the current state of fungal experimental evolution and why fungi are uniquely positioned to answer many of the outstanding questions in the field. We also review which fungal species are most well suited for experimental evolution.

Contents

1. What is experimental evolution?

The biological world we observe is the consequence of a single (and ongoing) evolutionary experiment. Observations are made to infer the evolutionary histories of populations and hypotheses are generated to explain these inferred histories and provide mechanistic insight into the evolutionary process. Yet testing specific hypotheses as to why evolution unfolded in a particular way is difficult because we cannot say anything about the countless other possible (but not realized) evolutionary histories for life on Earth. In his 1989 book, *Wonderful Life*, Stephen Jay Gould proposed the following thought experiment: rewind the tape of life and let evolution play out a second time. In doing so, does the replay produce anything like what we see today? In other words, is evolution reproducible, or do chance events (seemingly inconsequential at the time) cause evolutionary paths to diverge, producing wildly different outcomes? Gould himself favored a contingent world, arguing that the rapid emergence and decimation of biodiversity during the Cambrian period was seemingly random, dependent upon biological attributes that could not have been, *a priori*, identified as predictors of success. Gould asserts that "our origin is the product of massive historical contingency, and we would probably never rise again even if life's tape could be replayed a thousand

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times (Gould, 1989)." However, critics of contingency, such as Simon Conway Morris, point to convergent evolution as evidence that phenotypic evolution is strongly constrained (Conway Morris, 2003).

The balance between chance and determinism (between divergence and reproducibility of evolutionary paths) is a central issue in evolutionary biology. Disentangling the relative roles of chance and determinism through observation alone is difficult. In some fortuitous situations, natural replication allows one to follow evolution in several independent replicate populations. These "natural experiments" have been well studied, for example in Galapagos finches (Grant et al., 2004), *Astyanax* cavefish (Protas et al., 2006), and sticklebacks (Hohenlohe et al., 2010; Jones et al., 2012). These experiments suggest there is a degree of predictability in separately evolving populations facing the same selective pressures. Natural experiments, however, are not perfect replicates. Details of the environments will differ, the number of replicates is constrained, and experimental parameters typically cannot be tuned. Field studies are also constrained by incomplete characterization of ancestral populations, making it difficult to distinguish between ancestral variation and parallel evolution. Laboratory evolution experiments offer a complementary approach to studying the reproducibility of evolutionary outcomes, providing an opportunity to study adaptation in real time and in many replicate populations, with far greater control over experimental parameters.

At its core, the field of experimental evolution is the realization of Gould's thought experiment of "replaying the tape of life." With advances in high-throughput biology, we can perform Gould's thought experiment in the laboratory by initiating hundreds—or thousands—of initially identical populations to assess the full distribution of evolutionary outcomes given a set of initial conditions. Most bacteria, fungi, and nematodes can be cryogenically archived to generate "frozen fossil records" that can be returned to at any time in order to identify mutations, measure fitness, or replay the evolution experiment. Exchanging the complexities of the natural environment for the simplicity of the laboratory provides a number of additional advantages. Evolutionary parameters that are difficult to quantify in natural populations (such as population size and mutation rate) can be precisely measured and controlled. Selection can be tightly controlled by strictly defining media, temperature, and other growth conditions. Genetic variation can be defined at the onset of any experiment and gene flow can be absent or modulated. Population size and bottlenecks can be accurately quantified and kept constant, mitigating the effect of genetic drift. Gains in fitness can be tracked experimentally and high throughput next-generation sequencing can be applied to link changes in phenotype with underlying mutations. The large research communities devoted to the study of the model systems afford a number of tools for the genetic manipulation and the genetic and genomic analyses of evolved populations and provide a meaningful context in which to interpret the effects of individual mutations on fitness and other relevant phenotypes. Laboratory evolution experiments complement the study of natural populations, providing a system in which specific hypotheses can be tested. Furthermore, the replicate nature of experimental evolution can yield a wealth of information about the reproducibility and determinism underlying evolutionary processes.

2. A brief history of experimental evolution

The success of molecular biology in the last half of the 20th century was driven largely by model organism research. Evolutionary biologists were initially slower to exploit model systems, but recent adoption of model organism research has fueled a new understanding of fundamental aspects of the evolutionary process. Despite this recent and rapid growth, the field of experimental evolution traces its roots to the nineteenth century. The Reverend William Henry Dallinger (a contemporary of Charles Darwin) propagated three species of cytomonads (flagellated eukaryotes) for seven years, gradually increasing the temperature from 16 °C to 70 °C—beyond the normal limit of 60 °C (Dallinger, 1887). This experiment demonstrated that microorganisms could be used to study evolution over relatively short time scales. Throughout the middle of the 20th century, the extensive use of and dependency on microorganisms, primarily in the fields of molecular biology and biochemistry, set the stage for the development of experimental evolution. Two key events were the observation of clonal replacement within bacterial populations (Novick and Szilard, 1950; Atwood et al., 1951; Bryson and Szybalski, 1952) and the development of the chemostat for continuous culture (Monod, 1950; Novick and Szilard, 1950). The first applications of experimental evolution were as a tool to expand the metabolic repertoire of bacteria, for example to evolve novel amidase activity in *Pseudomonas aeruginosa* (reviewed in Clarke, 1983) or a second β-lactamase in *Escherichia coli lacZ* mutants (Campbell et al., 1973; Hall, 1977). These earlier studies were successful in demonstrating the potential for the evolution of novel biochemical and enzymatic functions, however they largely ignored the underlying evolutionary dynamics. The early studies on experimental evolution focused on the end products of evolution, not the process itself.

Evolutionary biologists soon began to leverage laboratory populations to address basic questions in evolutionary processes, particularly the tempo of adaptive evolution. Using chemostat cultures of *Saccharomyces cerevisiae* and *E. coli*, several groups determined the frequency of selective sweeps by monitoring the rise and loss of selectively neutral markers (Dykhuizen and Hartl, 1983; Paquin and Adams, 1983). This generation of evolution experiments addressed fundamental evolutionary questions such as the rate of adaptation in haploids versus diploids (Paquin and Adams, 1983) and the maintenance of variation (Helling et al., 1987). Chemostats have many advantages for experimental evolution such as the ability to define the limiting nutrient (and thus the selective pressure), and the ability to maintain a constant environment and constant population size. Today, the chemostat remains a powerful system for experimental evolution and is used in many laboratories (reviewed in Gresham and Dunham, 2014). However, chemostats are limited in their throughput and thus make it difficult to assess the reproducibility of evolutionary outcomes, and the need for growth-limited medium slows the growth rate and therefore reduces the number of generations that a chemostat evolution experiment can undergo. Batch growth—in which a fraction of each culture is used to seed fresh medium in repeated cycles of growth and dilution—is an attractive alternative. For batch growth experiments, the number of replicate populations and the total number of generations typically exceeds what is attained in chemostat evolution experiments. The longest running batch-culture evolution experiment began roughly three decades ago. In 1988, Richard Lenski initiated 12 replicate cultures of *E. coli*, which have been propagated daily for the last 28 years, surpassing 64,000 generations of growth. The Lenski long-term evolution experiment is the iconic example of laboratory experimental evolution. This experiment has led to a greater understanding of the evolutionary process, such as the role of mutator alleles in adaptation (Sniegowski et al., 1997), the tempo of genotypic and phenotypic adaptation (Barrick et al., 2009), the role of historical contingency in permitting adaptive evolution to acquire novel phenotypic traits (Blount et al., 2008), and the seemingly unlimited potential of adaptive evolution (Wiser et al., 2013). Many other bacterial and viral models have since been used in "evolve and transfer" experiments including *Pseudomonas fluorescens* (Barrett et al., 2005), *Methylobacterium extorquens* (Chou et al., 2011), *Myxococcus xanthus* (Velicer et al., 1998), and bacteriophage (Bull et al., 1997). With

each year, the list of model systems employed in the field of experimental evolution is growing along with the toolkit for analyzing laboratory evolution experiments (for recent reviews see Long et al., 2015; Schlotterer et al., 2015; Voordeckers and Verstrepen, 2015).

Each combination of model system and propagation method has its own advantages and disadvantages. The utility of the system is dependent on the types of questions to be asked. For example, outbred populations of the obligate sexual eukaryote, *Drosophila melanogaster*, have been effective for unraveling the role of standing genetic variation in adaptation, but because of the large amount of initial variation and the slow generation time (at least relative to microorganisms), *Drosophila* is not a good model for studying the role of *de novo* mutations in adaptation. Viruses, such as the ϕX174 bacteriophage, with kilobase-sized genomes, were for a long time the only system in which the full picture of the genomic response to selection could be monitored. Sanger sequencing methods, applied to experimentally evolved bacteriophage populations, provided the first genome-wide and population-level view of adaptive evolution in experimentally evolved genomes (Bull et al., 1997; Wichman et al., 1999; Bollback and Huelsenbeck, 2007, 2009).

Until recently such detail could not be attained for larger genomes; however, in the last several years, the decreasing costs of next generation sequencing and the decentralization of this technology have leveled the field. What once required large sequencing centers, can now be done in individual laboratories. The rapid advancement of sequencing technology has opened a new frontier in the study of experimental evolution. It is now practical and cost-effective to use whole-genome sequencing to analyze genetic changes in individuals or populations of laboratory-evolved organisms with much larger genome sizes such as bacteria (Barrick et al., 2009; Tenaillon et al., 2012; Maddamsetti et al., 2015), yeast (Kvitek and Sherlock, 2013; Lang et al., 2013; Kryazhimskiy et al., 2014), and *Drosophila* (Teotonio et al., 2009; Burke et al., 2010). Identifying mutations in laboratory-evolved populations is no longer limiting. In the last decade, whole-genome sequencing of laboratory-evolved populations has provided great insight into the mechanisms and dynamics of adaptive evolution by revealing *what* changes occur in evolving populations. The next decade in experimental evolution will focus on *why* particular mutations fix in a population and how the identity of fixed mutations affects subsequent evolution. Fungi—with fast growth rates, small genomes, and sexual cycles—are uniquely suited to this task. For fungal geneticists, a great opportunity awaits on the frontier of experimental evolution.

3. Current challenges and opportunities in experimental evolution

One of the key advantages of laboratory evolution is the ability to directly link fitness increases with underlying mutations in evolved populations, addressing fundamental questions about the relationship between individual mutations and overall fitness. Does the order in which mutations occur constrain evolution? Is it possible to predict how evolution will unfold by observing early steps along a particular path? If so, how far in the future can one forecast evolution? Answering these questions hinges on our understanding of genetic interactions, which alters the fitness effect of available mutations and thus the likelihood that evolution will favor one particular path over another.

The fitness effect of a particular mutation is dependent on the genetic background on which it arises. Epistasis (non-additive genetic interactions between mutations) is emerging as a common feature of genome evolution (Chou et al., 2011; Khan et al., 2011; Kvitek and Sherlock, 2011; Lang et al., 2013; Hong and Gresham, 2014; Kryazhimskiy et al., 2014). The study of epistasis between evolved mutations requires reconstructing each mutation in the ancestral background, individually and in combination with other mutations. This can be done in bacteria by transformation (Cooper et al., 2001, 2003; Crozat et al., 2005; Pelosi et al., 2006) or conjugation (Quandt et al., 2014) to replace individual ancestral alleles with evolved alleles, or vice versa. However, gene-by-gene reconstruction becomes prohibitively difficult as the number of mutations increases. Constructing all possible combinations of evolved mutations is far easier in fungal systems with a sexual cycle. For an evolved clone with n mutations, there are 2^n possible genotypes. Constructing all possible genotypes in a stepwise manner is impractical. This technical limitation has confined studies of epistasis in bacterial experimental evolution to five or fewer mutations (Chou et al., 2011; Khan et al., 2011). The power of fungal genetics, combined with the full functional genetic toolkit available to the fungal genetics community, provides a decisive advantage in constructing and analyzing all possible combinations of mutations from evolved populations. Long-term evolution experiments rarely yield just a handful of mutations, and fungal genomics techniques make it possible to study fitness and epistasis in evolved populations that have acquired dozens of mutations. Quantitative trait loci and bulk segregant mapping (Liti and Louis, 2012), barcode sequencing (Smith et al., 2009), and synthetic genetic arrays (Baryshnikova et al., 2013)—all tools piloted in the fungal genetics community—provide high-throughput methods for dissecting relationships between evolved mutations.

Sexual reproduction in fungal systems is advantageous not only because it allows genetic dissection of phenotypes, but also because the dynamics of adaptation in sexually reproducing populations remains largely unexplored. The nearly singular focus on asexual systems in experimental evolution is unfortunate: in nature, sexual reproduction is widespread and nearly all organisms participate in some sort of genetic exchange. In eukaryotes, sexual reproduction is regulated through a core program of cell-cell fusion (mating) and nuclear reduction (meiosis). This process is nearly ubiquitous: only two percent of eukaryotic species are asexual (Bell, 1982) and only a few taxonomic groups are ancient asexuals (Mark Welch and Meselson, 2000). The prevalence and conservation of sexual reproduction argues that it arose once, early in eukaryotic evolution (Goodenough and Heitman, 2014), and has therefore, played a central role in shaping the eukaryotic genome.

The prevalence of sex is nonetheless paradoxical given the many well-known costs to sexual reproduction (Bell, 1982; Otto and Lenormand, 2002; Otto, 2009). Most famous is two-fold cost of sex, first described by John Maynard Smith, which mathematically articulates the problem of sexual reproduction requiring two partners to produce one offspring (Smith, 1978). In addition, sex is energetically costly and slower than asexual reproduction (Otto and Lenormand, 2002). Hypotheses to account for the near ubiquity of sex despite these costs are varied, but generally invoke genetic recombination as the primary benefit—either to separate beneficial mutations from a deleterious load (Kondrashov, 1988; Peck, 1994), to produce favorable combinations of beneficial mutations, or to continually generate new genotypes to escape antagonistic interactions with parasites (Bell, 1982).

It has traditionally been difficult to directly examine the effects of recombination on fitness. This topic remains largely unexplored in the field of experimental evolution—and it is here that fungal model systems hold the most promise. Though sexual reproduction itself is ancient, fungi display great diversity in how it is carried out (from canonical mating and meiosis, to parasexual cycles, to asexuality) and how mating types are specified genetically (including tetrapolar, bipolar, and unipolar mating types) (Heitman et al., 2013; Sun and Heitman, 2015). The simple mating

and meiotic cycles of yeasts are beginning to be exploited to understand the dynamics of adaptation with recombination (Burke et al., 2010; Gray and Goddard, 2012; McDonald et al., 2016). The unique parasexual cycle in ascomycetes has been used to examine how mitotic recombination impacts adaptation in *Aspergillus* (Schoustra et al., 2007). A recent study in *S. cerevisiae* directly compared the rate of adaptation and the spectrum of evolved mutations in sexual versus asexual populations (McDonald et al., 2016). Sexual populations more effectively sort beneficial from deleterious mutations: sex combines beneficial mutations that arose in different lineages into the same genome, lessening clonal interference, and recovers beneficial mutations from deleterious backgrounds (McDonald et al., 2016).

The unique sexual cycles of fungi can be exploited to design experiments that study the processes underlying sex while inhibiting recombination. Homothallic sexual cycles allow examination of fitness effects of sex in the absence of recombination. Bruggeman et al. (2003) performed parallel mutation accumulation assays through transferring asexual or homothallic sexual spores and found that, despite absence of recombination, lines propagated via sexually produced spores showed higher average fitness than asexual lines. The significance of a diploid phase in sexual life cycles can be studied exclusive of genetic shuffling by evolving dikaryotic mycelia. Clark and Anderson (2004) found that experimental dikaryotic lines of *Schizophyllum commune* evolved faster relative growth rates than monokaryons while also exhibiting higher inter-population variance, suggesting diploid genotypes may have access to adaptive paths that are inaccessible to haploids. It is important to note that most hypotheses regarding sex are not mutually exclusive. It is likely that the forces driving the evolution and maintenance of sexual reproduction are diverse and nuanced. Combining these experimental approaches with genomics and quantitative genetics will elevate our ability to understand the evolutionary forces driving the prevalence of sex across life.

Experimental evolution in sexual fungi can be used to study the relationship between selection and reproductive isolation. For example, experimental evolution of isogenic *S. cerevisiae* populations under divergent selective conditions has been shown to generate intrinsic post-zygotic isolation in the form of reduced hybrid fitness (Dettman et al., 2007). Leu and Murray (2006) used experimental evolution to demonstrate that selection for assortative mating can reinforce species barriers. Experiments using *Neurospora crassa*, in which populations evolved under divergent selective conditions and populations evolved under parallel selection were crossed, found increased post-zygotic reproductive isolation following divergent selection (Dettman et al., 2008). Full integration of sexual reproduction into experimental evolution will make it possible to study a number of important problems that have been largely ignored in experimental evolution including speciation, the evolution of sex and recombination, and meiotic drive.

With the recent marriage of genomics and experimental evolution, the mechanisms by which complex traits respond to selection are beginning to be investigated. The most easily quantified complex trait in bacterial and unicellular fungi is fitness itself, however, studying the evolution of the discrete phenotypes under selection that contribute to observed gains in fitness presents more of a challenge. Phenotypic variation (other than growth rate) is difficult to detect and measure in unicellular models of evolution. Multicellular fungi provide more easily observable, complex phenotypes that can be used to model the interaction of trait space with selection. This approach to modeling the evolution of complex traits has been used in metazoans (McGuigan et al., 2005; Chenoweth et al., 2010), and has been applied to evolving lines of Aspergillus (Schoustra et al., 2012).

4. Tapping the power of fungal genetics

The potential of fungi as laboratory evolution models is due to their ideal position at the intersection of genomic simplicity, rapid life history, eukaryotic complexity and ecological diversity. Unlike metazoans and plants, most fungal genomes are compact. *Aspergillus* species range in genome size from 28 to 40 MB, the *Neurospora* genome is about 40 MB, and the *S. cerevisiae* genome is a tiny 12 MB. The largest of these is less than half the size of even the smallest model metazoan genomes of *Caenorhabditis elegans* or *D. melanogaster*. Fungal species have simple life cycles with rapid cell divisions. Many experimental evolution studies define generation as a single cell division, enabling many thousands of generations to be quickly achieved in the laboratory. Fungi exhibit the simple genomes and rapid cell cycles associated with other microorganisms while still retaining eukaryotic complexity that cannot be examined in experiments with bacteria. Another advantage is the diversity of ecological niches that fungal microorganisms occupy. Fungi, like bacteria, are amenable models for the study of drug resistance (Cowen et al., 2000; Schoustra et al., 2006). Pathogenic fungi are ideal models for studying pathogen-host coevolutionary dynamics (Forche et al., 2009; Vijendravarma et al., 2009), and the complexity of saprotrophic communities to which fungi belong can be exploited to study the relationship between competition and selection (Trienens and Rohlfs, 2011).

Fungal systems currently being used in experimental evolution include single-celled yeasts of the genera *Saccharomyces*, *Schizosaccharomyces*, and *Candida*, as well as the filamentous basidiomycetes *Schizophyllum* and *Ustilago*, and the ascomycetes *Neurospora* and *Aspergillus* (selected fungal experimental evolution studies are listed in Table 1). Many fungal models, including *Neurospora* and *Saccharomyces*, have been staples of genetics laboratories for decades. Fungi are advantageous in laboratory evolution experiments for a number of reasons. Many fungal species grow readily on agar media and are cryo-tolerant. Fungi generally have simple, rapid life cycles that can yield hundreds to thousands of generations over a relatively short period of time. Some species of fungi can be propagated as either haploid or diploid, sexually or asexually. Fungal crosses are easy to set up and progeny of crosses is easily isolated from parental cells. Large effective population sizes of 10^6–10^{10} cells (Gifford et al., 2011; Lang et al., 2011; Gresham and Dunham, 2014) are comparable to population sizes of bacterial experiments (Elena and Lenski, 2003) and are reflective of population sizes in nature (Tsai et al., 2008). Also like bacteria, growth rate can be a simple proxy for fitness, either through competitive growth assays in yeasts (Lang et al., 2009) or comparison of mycelial growth rate in filamentous species (Gifford et al., 2011). Conveniently, most fungal populations can be bottlenecked through a single cell to ensure genetic uniformity at the onset of an experiment.

Fully realizing the potential of experimental evolution with fungi requires tapping into the power of fungal genetics. A goal of most evolution experiments is linking phenotypic adaptation to causative genotypic changes. Next-generation whole genome sequencing has become the standard for examining whole genomes, however, the power of whole genome sequencing hinges on the availability of quality reference genomes. The heavyweight here by far is *S. cerevisiae*, the first eukaryote to have a complete genome sequence and arguably the best annotated eukaryotic genome. Other yeasts are closing the gap in terms of the availability of reference genomes and increased annotation, enabling experimental exploitation of the *Saccharomyces* species as a model genus (Hittinger, 2013). The Fungal Genome Initiative, commenced in November of 2000 by Gerry Fink, is credited with the wealth of fungal genome data that was produced throughout the decade

Table 1
Selected experimental evolution studies in fungi.

Fungal genus	Research focus[a]	Reference
Aspergillus	Distribution of mutational effects during adaptation	Gifford et al. (2011)
	Drug resistance and compensatory evolution	Schoustra et al. (2005) and Schoustra et al. (2006)
	Effect of mitotic recombination and alternation between ploidy states	Schoustra et al. (2007)
	Competition and co-evolution	Trienens and Rohlfs (2011)
Candida	Evolution of drug resistance	Cowen et al. (2001)
	Pathogen-host co-evolution	Forche et al. (2009)
Neurospora	Environmental uncertainty and bet hedging	Graham et al. (2014)
	Reproductive isolation and divergent selection	Dettman et al. (2008)
	Evolution of multicellularity	Bastiaans et al. (2016)
Saccharomyces[b]	Clonal interference	Kao and Sherlock (2008) and Lang et al. (2011)
	Adaptation to nutrient limitation	Ferea et al. (1999), Gresham et al. (2008), and Hong and Gresham (2014)
	Dynamics of genome sequence evolution	Kvitek and Sherlock (2013), Lang et al. (2013), and McDonald et al. (2016)
	Epistasis and distribution of mutational fitness effects	Kvitek and Sherlock (2011) and Kryazhimskiy et al. (2014)
	Parallel evolution	Segre et al. (2006)
	Reproductive isolation and divergent selection	Dettman et al. (2007)
	Ploidy and the rate of adaptation	Paquin and Adams (1983), Thompson et al. (2006), Gerstein et al. (2011), and Selmecki et al. (2015)
	Reinforcement and assortative mating	Leu and Murray (2006)
	Evolution of complex phenotypes	Ratcliff et al. (2012), Koschwanez et al. (2013), and Wildenberg and Murray (2014)
	Evolutionary recovery from deleterious mutations	Rancati et al. (2008) and Laan et al. (2015)
	Evolution from standing genetic variation	Burke et al. (2014)
Tubulinosema	Pathogen-host co-evolution	Vijendravarma et al. (2009)

[a] Primary research focus. Many papers address multiple topics.
[b] For *Saccharomyces*, where experimental evolution has been widely used, this list is intended to be representative, not comprehensive.

following the sequencing of *S. cerevisiae*. The *N. crassa* genome (Galagan et al., 2003) along with the *Aspergillus fumigatus* genome (Nierman et al., 2005) shortly followed a formal prioritization of fungal species for sequencing (Birren et al., 2003). Genome sequence availability is no longer a limiting factor in fungal experimental evolution and the wealth of post-genomics era fungal genome data is curated on FungiDB along with genus-specific databases (Stajich et al., 2012). The next step in tapping the power of fungal genetics is to begin to leverage fungal genome sequences as tools in the analysis of experimental outcomes.

Determining causal relationships between *de novo* mutations and gains in fitness requires more than the simple identification of evolved mutations; it requires the dissection of mutational combinations in tandem with fitness assays. Here, high throughput techniques that permit rapid mutant construction are crucial. Directed gene replacement and efficient marker rescue techniques are routine in diverse yeast species such as *Saccharomyces*, *Candida*, and *Schizosaccharomyces*, as well as filamentous species such as *Aspergillus* and *Neurospora*. CRISPR/Cas9 technology is on pace to eliminate the need for sequential allele replacements in yeasts with single step simultaneous gene modifications (Mans et al., 2015). While methods of genotype construction are important in achieving a detailed picture of epistasis within a genome, adoption of high throughput methods to survey functional genetic variation can be a powerful tool for characterizing of the distribution of mutational effects in evolving populations. Quantitative trait loci and bulk segregant mapping methods have been used to link genotype to phenotype in yeasts, particularly *Saccharomyces* and *Schizosaccharomyces* (Liti and Louis, 2012; Hu et al., 2015). These methods can be applied to other fungal species, many of which have genetic linkage maps (Foulongne-Oriol, 2012). For example, exploitation of parasexual cycles is used to conduct bulk segregant analysis in the homothallic fungi *Aspergillus* (Niu et al., 2015). The broad application of quantitative genetics to map evolved traits in fungi makes possible the detailed study of the genomic basis of adaptation in laboratory populations of fungi.

In the last several decades we have witnessed rapid growth in the field of experimental evolution. In fungi, most of this work has focused narrowly on a few species, most notably the yeast *S. cerevisiae*. The relevant traits—small genomes, rapid growth, existence of sexual cycles, and amenability to quantitative genetics—are shared among many fungal species. Moreover, these species show diversity in life histories. Experimental evolution in fungi has the power to provide a deeper understanding of evolution. In turn, studying how fungal genomes respond to selection will provide new insights into fungal biology, furthering their significance as model systems and opening new research directions. Fungal diversity is an untapped resource for the field of experimental evolution. Efforts to incorporate more diverse species into laboratory evolution experiments will increase our understanding of both evolution and fungal biology.

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