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The fitness of filamentous fungi

Anne Pringle and John W. Taylor

Fitness is a common currency in comparative biology. Without data on fitness, hypotheses about the adaptive significance of phenotypes or basic mechanisms of evolution, for example natural selection, remain speculative. Experiments with fungi can address questions specific to fungi or questions with a broader significance. Fungi can challenge the generality of fundamental evolutionary principles, yet there are no standard measures of fungal fitness. We argue that focusing on a single aspect of a complex life cycle, or a single measure of fitness (e.g. the number of asexual spores) is appropriate. Choosing which aspect of fitness to measure can be facilitated by an understanding of how fitness measures are correlated. Choices can also be based on the ecology of a species, for example whether a fungus is semelparous and reproduces once, or iteroparous and reproduces multiple times.

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There are at least two reasons why we should try and understand the fitness of filamentous fungi. The first is to use data from the third kingdom of complex multicellular eukaryotes to challenge our understanding of processes as diverse as the evolution of sex, natural selection and the ubiquity of Mendelian genetics (Box 1). The unique features of fungi can provide answers to basic questions that cannot be addressed using animals or plants, such as the ubiquity of meiotic drive in nature [1]. In fungi, the products of a single meiosis can be bound together, for example in species of *Neurospora*, and so in principle all instances of meiotic drive within an individual, and interactions between elements of meiotic drive, can be measured; this experiment is impossible using animals or plants. A second reason to understand the fitness of filamentous fungi is to explore those questions that are specific to fungi, especially questions concerned with the prevention and treatment of disease (Box 2). Mycologists build phylogenies to define species and describe populations [2,3], but a complete understanding of fungal evolution requires an understanding of how fitness shapes the selection of individuals in nature. This understanding will require the active manipulation of fungal individuals in the laboratory and field; as Anderson and Kohn [4] have stated 'the next wave of research will be experimental'. This claim is substantiated by the experimental work of Cowen *et al.*, who use fitness to explore drug resistance in *Candida albicans*, a fungal pathogen of humans [5].

Fitness in budding yeasts is routinely measured by counting cells. Most fungi, however, are filamentous;

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Box 1. Important unanswered questions: an evolutionary perspective

- How does natural selection operate? A recent review of phenotypic selection in the wild [a] did not include any examples of fungi. However, experiments are possible – for example, Brasier [b] showed selection for intermediate growth rates in a natural population of *Schizophyllum commune*. Furthermore, genetic markers have been used to describe natural selection in fungal populations [c]. Fungi might also be used to reconcile questions related to the various levels of selection [d]. For example, *Aspergillus flavus* might suppress recombination between the multiple genes of the aflatoxin pathway to connect selection of a genotype directly to selection of the phenotype.
- Why are organisms asexual [e]? How do asexual lineages establish, flourish, dominate a community or evolve to novel 'species'? Comparisons of the fitness of asexual versus sexual lines in fungi (in a variety of environments) might answer these questions. The subject has been usefully explored using yeast [f,g], but not filamentous fungi. Unlike yeast, many of the stable asexual and sexual lineages of filamentous fungi are found in nature; for example, sexual and asexual clades of *Aspergillus nidulans* coexist within an Australian field [h]. Furthermore, well-studied filamentous fungi, for example *Neurospora crassa* and *Aspergillus niger*, might be used to test specific hypotheses related to the evolution of sex. For example De Visser *et al.*[i] used *A. niger* to test an hypothesis that sex facilitates the purging of epistatic and deleterious mutations.
- A variety of non-Mendelian genetic phenomena can be investigated with fungi, including transmission-ratio distortion. For example, *Neurospora* 'spore killer' strains maintain an allele that is lethal to any spore without the spore killer allele [j]. Crosses between spore killer and sensitive strains yield fruit bodies in which half of the spores (those without the spore killing allele) are killed, exactly analogous to the t haplotype of mice. Other segregation distorters have been found in other fungi [k].
- How do mutualisms limit or tolerate exploitation? Many classic mutualisms involve filamentous fungi, for example, mycorrhizas and lichens. Determining the costs and benefits of any association requires measurement of fungal fitness with a wide array of symbionts (A. Pringle, unpublished).
- How does virulence evolve? How are virulence and fitness related? Filamentous fungal pathogens are devastating to crops and cause serious animal and human diseases, and understanding the evolution and ecology of these pathogens would meet a particularly poignant need.
- A variety of filamentous fungi are plant pathogens, and the costs of resistance to fungicides have been well-described in the literature of these fungi (for a recent example see the work of Raposo *et al.*[l] on *Botrytis cinerea*). Experiments with yeast show that such costs are temporary [m]. The same experiments have not been completed with filamentous fungi.
- What are the advantages of various mating systems? Closely related lineages of fungi can be entirely outbreeding or inbreeding, for example, heterothallic species of the genus *Neurospora* are outbreeding whereas homothallic species are inbreeding. Fungi can also possess mixed mating systems. Inbreeding depression has been well-described for *Agaricus bisporus* [n]; outbreeding depression has been described for isolates of *Aspergillus nidulans* [o].
- As Mather and Jinks [o] wrote, fungi offer 'the unique possibility of comparing the actions of the same genes in the monokaryotic (haploid) and dikaryotic (effectively diploid) conditions'. Levels of ploidy might also influence the speed of adaptation (Clark and Anderson, pers. commun.)

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aspects complicating the measurement of survival and reproduction in filamentous fungi include confusing definitions of the 'individual' [6], and life cycles and genetics that seem complicated or unusual when compared with animals or plants (Box 3). For example, fungal filaments can differentiate to produce mitotic spores (asexual spores or conidia) or mate and produce meiotic spores (sexual spores: ascospores or basidiospores). Fitness can be assessed by measuring growth, or either mitotic or meiotic sporulation. Sexual forms of fungi are often geographically restricted but asexual clones can propagate from a sexual center and dispersal is often facilitated by agriculture or other human activity. Before fitness is measured for a specific population, mycologists must understand whether that population is asexual, sexual or both.

In this review, we hope to elucidate the problems associated with understanding the fitness of filamentous fungi by making three points. First, a focus on a single stage of a complex fungal life cycle is appropriate; this has been amply demonstrated by plant pathologists. Second, choosing to measure a single aspect of fitness, for example mycelial growth, is useful especially if the choice is guided by an understanding of how measures of fitness are correlated. Finally, the distinction made by botanists between plants that reproduce once or multiple times can also be used by mycologists to classify species of fungi and choose between different estimates of fitness.

Fitness concepts

Fitness can be defined as the survival and reproductive success of an allele, individual or

Box 2. Important unanswered questions: a mycological perspective

- Recent data show that gene flow in even cosmopolitan species of fungi is locally restricted [a], but mushroom spores are globally dispersed; furthermore, geographically distant isolates can mate in the laboratory. If spores are globally dispersed, what mechanisms cause spatial structuring? How are fungal populations established in novel habitats, or kept from establishment?
- Are local populations 'adapted'? Useful definitions of fitness would facilitate 'common or garden' experiments designed to explore the ecological niches of different genotypes of a species. Surprisingly, results can show that fungi are not 'adapted' – see Ennos and McConnell's study of the canker pathogen *Crumenulopsis soraria* [b].
- How does fungal resistance to fungicides evolve? A working definition of fitness might aid medical mycologists' abilities to describe the origin, and especially invasion dynamics, of resistant mycoses [c]. In agriculture, a better understanding of fungal evolution might aid in the active manipulation of the evolutionary process, allowing, for example, crops resistant to fungal diseases to remain resistant [d].
- Exploring the fitness of various life history stages could facilitate mycologists' understanding of complex life histories: for example, the ubiquity with which fungi create multiple kinds of sexual and asexual propagules (Fig. 1). Few data are available to judge even the most basic hypotheses, for example, that mycelial growth allows for invasion within a habitat but sporulation allows for dispersal from a habitat.
- Why do species of fungi maintain vegetative incompatibility groups (VIGs) [e], which prevent the fusion of genetically different individuals? Are VIGs a barrier to viral diseases, as evidence from *Ophiostoma novo-ulmi* [f] would suggest?

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Box 3. Aspects complicating measures of fungal fitness

- What is an individual? Mycologists must understand the physical limits of a single organism before that organism's fitness can be measured. Evidence for gargantuan clones of *Armillaria* [a,b] left many biologists with the sense that defining the physical individual is a hopeless task. But in fact the majority of fungal individuals occupy a much smaller space, for example, genets of the ectomycorrhizal fungus *Amanita francheti* range from 1.5 to 4.7 m^2 [c] and genets of the pathogen *Ophiostoma novo-ulmi* are measured in centimeters [d]. Many plant pathogens are restricted to a single host, for example, individuals of the smut *Microbotryum violaceum* are usually restricted to a single *Silene alba* plant. Lichens grow on exposed substrates where an individual lichen is easily identified.
- The genetical systems of fungi encompass a dazzling array of unusual phenomena, including reports of the sorting of distinct nuclei into different spores such that asexually derived spores are genetically variable [e,f] and parasexuality, in which unlike nuclei fuse and give rise to a recombinant nucleus without meiosis. As Hoekstra [g] has discussed, even a minimal level of parasexuality would radically alter the genetic structure of a population. Fungi also maintain segregation distorters, B chromosomes, unusual levels of chromosomal polymorphisms [h] and mechanisms by which duplicated sequences, including transposable elements, are destroyed (e.g. the RIP mechanism of *Neurospora* [i]). Asexually propagated cultures of fungi are extraordinarily labile, for example shifting from mitosporic to mycelial growth forms in an apparently non-Mendelian manner [h]. An understanding of individual fitness will be most useful when the understanding is contextualized by a clear sense of the genetical systems of fungi.
- Recent data show that mushrooms of *Armillaria gallica* are genetic mosaics [j]. The significance of these data are unclear. If the data are confirmed, they will complicate efforts to define the fitness of individuals of *A. gallica*, and perhaps other mushrooms. The biology is analogous to the reproductive habits of cellular slime molds [k], in which fruit bodies can be formed from genetically distinct individuals.
- Fitness can be measured with either asexual or sexual propagation. Commonly, clonality is equated with asexuality, and recombination with sexuality. However, haploid fungi that self-fertilize behave clonally, even if they have a sexual morphology and meiosis. Conversely, mitotic spores can function as male fertilizing elements, akin to sperm, and not as agents of clonal reproduction, as in *Neurospora* species. The same fungal individual often reproduces clonally as well as by recombination, and in some species, such as rusts, the reproductive mode is correlated with plant host. However, fungi capable of both modes of reproduction do not always use both. Plant pathogenic fungi can be exclusively clonal in association with agricultural hosts, but recombine in other, geographically restricted areas; a well-studied example is *Sclerotinia sclerotiorum* [l].
- Fungal species are usually recognized by their morphologies, but recent phylogenetic analyses have shown that morphospecies can contain two or more genetically differentiated groups (*Coccidioides immitis* [m] is divided into two phylogenetic species, *Aspergillus flavus* [n] into four). If this information is unavailable, measures of fitness might be confounded by the inadvertent comparison of individuals of different species.

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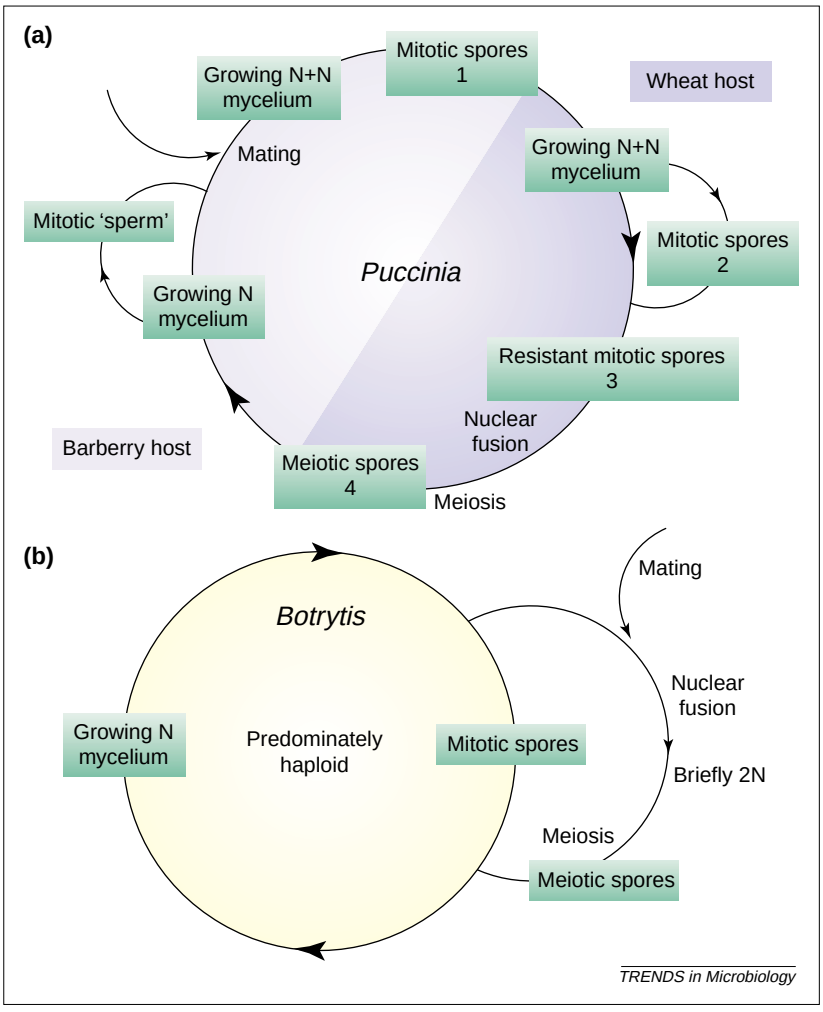

Fig. 1. The life cycles of two fungal pathogens. (a) *Puccinia graminis* and (b) *Botrytis cinerea*. Features that can serve as measures of fitness are boxed.

group [7]. Population biologists have generally focused on the absolute fitness of individuals within populations, for example, the number of seeds made by a plant. The measure of fitness, R, is formally equivalent to:

$$R = \sum l_x m_x \qquad \text{[Eqn 1]}$$

where l_x is the probability of survival to age x and m_x is the number of progeny produced by an individual of age x. Measuring R is laborious and instead population biologists might measure various indicators of individual fitness, such as the mortality, size or weight of plants. Many of the questions outlined in Box 1 are best answered using measures of individual fitness, but measuring individual fitness requires that mycologists define an individual. A colony of *Penicillium* growing on a lemon is easily described, but a mycorrhizal fungus growing in forest soil is more difficult to measure. As has been noted (in Worrall [8] and references therein), the concepts of ramet and genet will be especially useful: as a mycelium fragments, its pieces become ramets of the same genet. Ramets can propagate indefinitely, and for this reason the genet can be immortal [9], but only if somatic mutations are rare. Different experimental questions will require divergent foci. For example, questions related to the importance of asexual reproduction might require that individual ramets are counted, however, questions related to the genetic diversity of a population will require individual genets are counted. The fitness of an individual genotype can also be measured by choosing a single spore and using the asexually derived progeny of that spore to measure a specific aspect of fitness, for example mycelial growth rate [10]. The various indicators of fitness can be correlated and, as illustrated by Xu [11], the correlations can be measured before choosing one particular indicator for an experiment. Other aspects of fitness might involve negative correlations, or trade-offs; for example, a genotype or species might invest limited resources in either mycelial growth or sporulation. Arbuscular mycorrhizal fungal sporulation might involve a trade-off in number and size; species create either limited numbers of large spores or numerous small spores (J. Bever and M. Allen, pers. commun.).

Fungal populations often include individuals of different sizes or ages. The survival and reproductive success of individuals will vary with age, and measures of fitness should consider this variation [12]. For example, early reproduction might have a greater impact on total fitness than late reproduction: a single genet that can establish quickly in a novel habitat will overwhelm a second genet that establishes slowly, even if the propagation rates of the two genets are eventually equivalent. The fecundity of filamentous fungi can also be correlated to the size of an individual (discussed later); in either case, measures of fitness should be taken with an understanding of how the size or age of the measured individual might influence its absolute fitness.

Choosing a single measure of fitness requires assumptions about how that measure is influenced by natural selection, but assumptions related to the fitness of plants or animals might be irrelevant to fungi. For example, in plants and animals, smaller progeny are assumed to be less fit than larger progeny. But in fungi smaller spores can be dispersed more effectively and therefore be more fit than larger spores, even if larger spores have greater germination rates; stabilizing selection can optimize spore size [13].

A more appropriate measure of fitness for some microorganisms, and perhaps fungi, is Malthusian fitness (M), or the instantaneous growth rate of a genotype [7]. Malthusian fitness measures the growth rate of a population, is commonly used for bacteria and is usually measured by growing individuals in artificial media and comparing the number of individuals at an initial time, N_0, to the number of individuals at a future time, N_t:

$$M = \frac{\ln(N_t/N_0)}{t} \qquad \text{[Eqn 2]}$$

Malthusian fitness can be equated to the number of cell doublings by dividing by ln(2). To our knowledge, Malthusian fitness has not been used to measure the fitness of filamentous fungi, but this technique could be useful. An accurate comparison of the fitness of two different genotypes could be made by using spores of each genotype to start colonies in separate petri dishes. The number of spores in each dish would be measured after a defined amount of time and used to calculate a Malthusian fitness.

Our discussion focuses on individual selection. Historically, plant pathologists have considered group rather than individual selection [10]. In fact, group measures of fitness might be appropriate for species of fungi distributed in spatially structured and genetically related groups, for example animal mycoses [14]. For more on group selection in fungi, see Antonovics and Alexander [10].

Focusing on a single aspect of a complex life cycle

Fungi infect every kind of wild and crop plant. Plant pathologists use fungal population biology, especially population genetics, to address questions related to the evolution of disease in agriculture [15,16]. These mycologists are especially concerned with the evolution of virulence and the emergence of fungal resistance to fungicides. In addition to the usual measures of growth and sporulation, pathologists can assess the ability of individual fungi to cause disease. Pathogens can have complex life histories and no single measure of fitness can be applied to all species. But plant pathologists normally make the choice to study one aspect of a complex life cycle, and this focus can elucidate a great deal of useful biology. The fundamental biology of pathogenic and other fungi is the same and so approaches used by pathologists to measure fitness can be adopted by other mycologists. We will consider two examples: the rust fungus *Puccinia graminis* and the grey mold *Botrytis cinerea*.

Puccinia graminis*: a fungus with a complicated life history*

Puccinia graminis f.sp. *tritici* is the wheat rust fungus. This pathogen infects two hosts (wheat and barberry) and creates five distinct kinds of spores (the first establishes on wheat; a second is created on and reinfects wheat; a third overwinters; the fourth is the meiotic spore and infects barberry; and a fifth, formed on barberry, functions as sperm on *Puccinia*; Fig. 1a). Urediniospores are the mitotic propagules that infect and spread the rust disease on wheat. The lesions caused by urediniospore formation are easy to measure, and urediniospores can be counted. A focus on the fitness of this one morph of *P. graminis* has allowed plant pathologists to understand the epidemic dynamics of rust disease on wheat; for example, data collected by Newton *et al.* [17] demonstrate that strains of *P. graminis* adjust the timing of urediniospore production according to the density and kind of fungal individuals in their environment. The latent period is minimized when initial densities are greater or if competitors are present. The result has an important theoretical implication: Lehman and Shaner [18] have demonstrated that strains of a related species, *Puccinia recondita*, with shorter latent periods cause significantly more disease than strains with longer latent periods. Furthermore, the latent period is a heritable trait [18] and can be manipulated via artificial selection [19].

This result could be a general one: experiments with other fungi have also shown that total spore production might be less important to absolute fitness than the length of a latent period [10,20]. In fact, Kato *et al.* [21] showed that an isolate of *Phytophthora infestans* common in the United States before 1991 was replaced by isolates with a shorter latent period. The advantages of early reproduction (discussed earlier) have been carefully explored by plant ecologists [22]; apparently the same advantages operate in fungal communities.

Other studies with rust urediniospores have shown that a strain judged to be less competitive when grown alone can possess a relative fitness advantage when strains are mixed, if that strain also displays a greater ability to tolerate crowding or carrying capacity [23]. Once again, this result could be a general one: data from *C. albicans* indicate that the fitness of a fungus grown in isolation might not predict its fitness in competition with other genotypes [5]. In addition, studies with rust urediniospores have tracked the changes in populations infecting barley as being caused by differences in competitive ability [24] and demonstrated that in *P. recondita* unnecessary virulence genes might not incur fitness costs [25,26]. Although a complete understanding of the ecology of the species might be limited by the focus on a single aspect of the *P. graminis* life cycle, in fact, this approach has elucidated a great deal of useful biology.

Botrytis cinerea*: a predominately asexual fungus*

Botrytis cinerea causes the 'grey mold' disease of a wide variety of fruits, vegetables and ornamentals, and the 'noble rot' of grapes. *B. cinerea* is a cosmopolitan fungus and an economically devastating pathogen in temperate climates. The disease is successfully thwarted by treatment with fungicides [27], and plant pathologists are motivated to understand how fungicide resistance evolves and how resistance might be managed. *Botrytis cinerea* is capable of meiotic and mitotic reproduction, but meiosis is rarely observed [28] (Fig. 1b). As a result, plant pathologists have focused on understanding the evolution of resistance via measures of growth and mitotic spore production. For example, Ziogas and Kalamarakis [29] explored the costs of resistance by culturing *B. cinerea* strains and testing for a variety of fitness parameters including the radial growth of mycelia on petri dishes, spore germination, the mean length of germ tubes and pathogenicity (the infection caused by each strain on a naive plant). In at least one

case, a fungicide-resistant pathogen was as virulent as the wild-type strain [29], demonstrating that chemical resistance might not impose a fitness cost. As the population genetics of this species is more carefully explored [28], a more refined understanding of the evolution of resistance will be possible. In the meantime, accurate assessments of whether and when resistant strains will evolve, and be fit enough to survive in the wild, are possible because of the focus on the asexual stage, as is the development of successful management strategies [27].

A similar approach has been developed in the sugar-beet leaf-spot *Cercospora beticola*. Like *B. cinerea*, it is capable of mitotic and meiotic reproduction, but only mitospores are seen in the field. Again, pathologists have focused on mitotic reproduction and this strategy has been useful. For example, a recent study described both predicted and realized fitness by first measuring (among other parameters) mycelial growth, spore germination, germ-tube length and spore production, and subsequently conducting competition experiments between isolates [30]. Fungicide-resistant isolates were slightly less competitive than sensitive isolates, suggesting that chemical resistance does impose a fitness cost in this fungus.

In each of these examples pathologists have focused on a single aspect of the fungal life cycle and this focus has facilitated a greater understanding of the disease. This strategy could prove useful to other mycologists, especially in the many cases where it is impossible or impractical to record data on every aspect of an organism's biology.

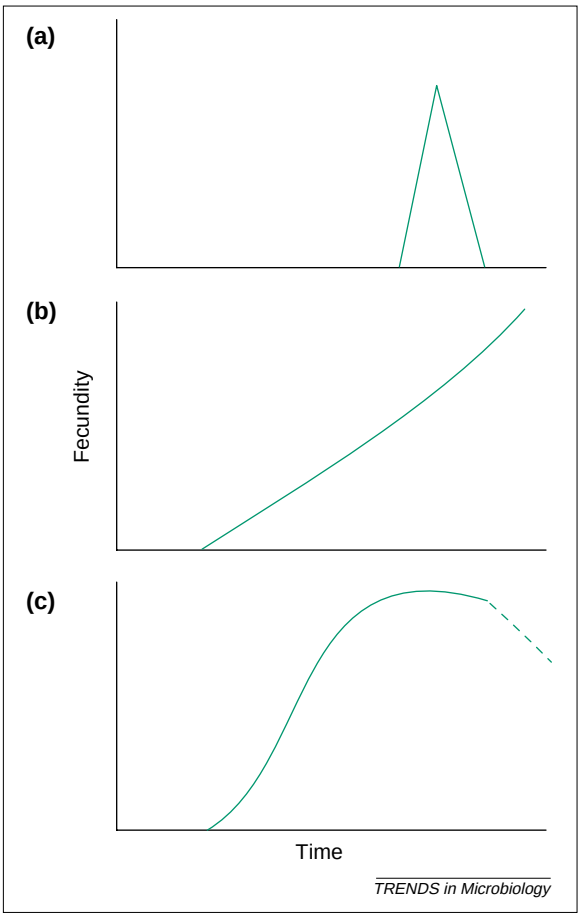

Fig. 2. The reproductive schedules of (a) semelparous fungi and (b) clonal or (c) aclonal iteroparous fungi. Adapted from [44] with permission.

Correlating measures of fitness

Fitness is studied less often in non-pathogenic fungi for obvious economic reasons, but some of the most careful work has been done with the cultivated mushroom *Agaricus bisporus* [11].

In a study published by Xu [11], different measures of fitness were compared. Measures included mating behavior (if two mycelia were compatible or antagonistic); mating success (if two compatible mycelia fused to form a heterokaryon); growth rate of a newly formed heterokaryon; days to the first appearance of a fruit body; whether or not primordial or mature mushrooms were formed; number of fruiting bodies per unit area; and average weight of fruiting bodies. In fact, only two sets of measures were correlated: mating behavior was a significant predictor of mating success, and days to fruiting was a significant correlate of the number of fruiting bodies per unit area. Although the growth rate of mycelia was not by itself a predictor of sexual success, it was significantly minimized in inbred populations of *A. bisporus* and thus was a useful measure of inbreeding depression. These data are a rare example of how different measures of fitness can be compared and correlated. With these data, it is possible to measure one aspect of fitness in *A. bisporus* and understand how that measure relates to other aspects of fitness. It would be useful to have similar data for other species of fungi.

Often a mycologist does not know how different aspects of fitness are correlated within a given species, and then which aspect of fitness should be measured? Mycelial growth rates are easy to measure and can be used to explore many crucial questions. Brasier [31] has called these 'under-utilized… [but] the most available analytical tool in fungal biology' and they have been used to measure epistasis in haploid fungi [32]. A correlate of mycelial growth rate, mycelial surface area, can also be used to predict fitness. An experiment with *Aspergillus niger* showed that mycelial surface area is a significant predictor of spore number [32]. An analogous measure of fitness, the functional surface area of fruiting bodies, was used to gauge the impact of fungivory in a natural population of *Coriolus versicolor* [33].

Natural selection in filamentous fungi

To measure natural selection, data must be collected from individuals followed through time and measured for multiple fitness parameters (e.g. survival or number of matings) [34]. These kinds of experiments are a practical impossibility for most fungi, at least in

nature, because fungal individuals are usually difficult to recognize. As a result, a recent review of selection in the wild [35] included no examples of fungi. However, lichens might be used to evaluate selection in nature because individual lichens are exposed on rocks or other substrates and an individual lichen is easily delineated. Sanders [36] marked thalli for long-term developmental studies, and evolutionists could do the same. Furthermore, the meiotic structures of lichens (called apothecia) are permanently bound to the lichen thallus, and so the lifetime fecundity of individual lichens can be measured (A. Pringle, unpublished).

Demonstrating natural selection in the field can be difficult, but laboratory manipulations are possible. A wonderful experiment by Brasier [37] showed selection for intermediate growth rates in a natural population of *Schizophyllum commune*. Wild isolates were collected and mated. Progeny growth rates showed extremes not seen in wild individuals, evidence of strong stabilizing selection in nature.

Laboratory populations can also be artificially selected to explore the genetic variation of a character or the potential speed of selection. For example, both growth rate and spore size have been manipulated in *Neurospora crassa* [38,39], growth rate has been manipulated in *S. commune* [40], and in pathogens, the latent period of *P. recondita* [19] and the lesion size of *Cochliobolus heterostrophus* [41] have been artificially evolved.

Genetic markers can be used to track the survival of individual alleles or as proxies for the success of various genotypes; a recent review discusses the use of genetic markers in studies of natural selection [42]. Ennos and McConnell [42] show that selection coefficients can be very large, for example a favored genotype can be five times more fit than an unfavored genotype. However, the majority of the studies evaluated selection within plant pathogens, for example selection for fungicide resistance, and the bias is likely to inflate coefficient values because fungicides impose artificially strong evolutionary pressures. Nonetheless, the data indicate that selection coefficients are likely to be: (1) significantly greater than zero; (2) larger when selection on genotypes (as contrasted to selection on alleles) is considered; and (3) strongly dependent on the environment.

Sexual selection, or selection on variation in mating success [34], is rarely described for fungi. But individual fungi clearly vary in their abilities to mate [43]. Furthermore, as fungi can be hermaphrodites, sexual selection can act on an individual's success as a male or female (i.e. ability to conidiate or accept conidia in species of *Neurospora*).

Fungi can be classified as either semelparous or iteroparous

Plants can be characterized by the number of reproductive events experienced by an individual in a lifetime, and the same dichotomy can be applied to fungi. Plants that reproduce once are annual, or semelparous, and plants that reproduce more than once are perennial, or iteroparous [44]. Because almost all plants are autotrophs, the difference between semelparous and iteroparous species has little to do with energy availability, however, all fungi are heterotrophs and whether or not a fungus is semelparous or interoparous depends on whether or not its energy source is ephemeral. Semelparous fungi exploit ephemeral foods and a semalparous fungus can complete its life cycle in a few days or weeks, for example, species of dung fungi (e.g. *Coprinus* or *Pilobolus* spp.), fungi that spoil food (e.g. *Penicillium italicum* on a lemon), or fungal pathogens of small animals (e.g. *Batrachochytrium dendrobatidis* on frogs). Iteroparous fungi exploit more stable foods and include mycorrhizal symbionts and decomposers of forest trees.

The reproductive schedules of semelparous and iteroparous individuals are very different (Fig. 2a–c). The fitness of semelparous plants is routinely measured by growing the plant from seed to seed in a pot; the fitness of semelparous fungi might also be measured by growing the fungus from spore to spore in a petri dish, and perhaps estimating a Malthusian fitness.

Iteroparous fungi can be divided into species whose ramets are physiologically independent (Fig. 2b) and fungi whose growth maintains a single physiological individual (Fig. 2c). In the first case a genet is potentially immortal [9]; as ramets propagate and the genet increases in size the probability of genet death decreases, if ramets experience mortality risks more or less independently [45]. Individual ramets will propagate further ramets and fecundity is unlimited. In the second case senescence will eventually limit new births. A complete understanding of the fitness of an iteroparous fungus might require a dual approach focused on both the survival of individual genets and the births and deaths of each genet's ramets [45]. As we have discussed, different questions will require divergent approaches.

Conclusion

Fungi are complicated, but tractable. Aspects complicating measures of fungal fitness, for example complex life cycles and cryptic sexuality, can be circumvented by the careful choice of ecologically relevant fitness parameters; this has been done by plant pathologists working with *P. graminis* and *B. cinerea*. A single measure of fitness, for example mycelial growth rate, can be used to estimate fitness, especially if the choice is guided by an understanding of how measures of fitness are correlated, as is possible with *A. bisporus*. The biology of a species might facilitate the choice of a fitness measure, for example, if the species is a semelparous fungus, spore numbers could be the logical choice. Carefully

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stated assumptions are necessary. Different experiments can use different measures of fitness, for example, mycologists working with arbuscular mycorrhizal (AM) fungi must choose between measuring hyphae or counting spores: choosing hyphae as a measure of fitness showed that a species of AM fungus can grow equally well with a variety of plant species [46]; choosing sporulation illustrated that ecological feedback might promote the diversity of fungal and plant populations [47] (for more on sporulation as a measure of AM fungal fitness, see Bever [48] and Olsson *et al.* [49]). Fungi can be used as models by evolutionists, and an understanding of fungal evolution, and especially the natural selection of fungi, will be enhanced by careful experimentation.

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