Synthesis

The Case of the Missing Ancient Fungal Polyploids

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abstract: Polyploidy—the increase in the number of whole chromosome sets—is an important evolutionary force in eukaryotes. Polyploidy is well recognized throughout the evolutionary history of plants and animals, where several ancient events have been hypothesized to be drivers of major evolutionary radiations. However, fungi provide a striking contrast: while numerous recent polyploids have been documented, ancient fungal polyploidy is virtually unknown. We present a survey of known fungal polyploids that confirms the absence of ancient fungal polyploidy events. Three hypotheses may explain this finding. First, ancient fungal polyploids are indeed rare, with unique aspects of fungal biology providing similar benefits without genome duplication. Second, fungal polyploids are not successful in the long term, leading to few extant species derived from ancient polyploidy events. Third, ancient fungal polyploids are difficult to detect, causing the real contribution of polyploidy to fungal evolution to be underappreciated. We consider each of these hypotheses in turn and propose that failure to detect ancient events is the most likely reason for the lack of observed ancient fungal polyploids. We examine whether existing data can provide evidence for previously unrecognized ancient fungal polyploidy events but discover that current resources are too limited. We contend that establishing whether unrecognized ancient fungal polyploidy events exist is important to ascertain whether polyploidy has played a key role in the evolution of the extensive complexity and diversity observed in fungi today and, thus, whether polyploidy is a driver of evolutionary diversifications across eukaryotes. Therefore, we conclude by suggesting ways to test the hypothesis that there are unrecognized polyploidy events in the deep evolutionary history of the fungi.

Keywords: whole-genome duplication, polyploidy, paleopolyploidy, fungi, chromosome number evolution, diversification.

Introduction

Polyploidy, the increase in the number of whole chromosome sets, is an important evolutionary force observed throughout the eukaryotic domain of life (Otto and Whitton 2000; Mable 2003; Albertin and Marullo 2012). Polyploidy results from increases in chromosome sets due to whole-genome duplication (autopolyploidy) as well as mergers between different species (allopolyploidy) and can result in instantaneous speciation. It creates opportunities for adaptive evolution by providing high levels of genetic redundancy (Otto and Whitton 2000; Mable 2003; Comai 2005; Albertin and Marullo 2012) and is well documented in plant and animal evolutionary histories (Thompson and Lumaret 1992; Otto and Whitton 2000; Taylor et al. 2003; Blomme et al. 2006). Polyploidy has been linked to major evolutionary radiations (Comai 2005; Van de Peer et al. 2009) and has been proposed to help adaptation to stressful environments (Vanneste et al. 2014*a*, 2014*b*; Soltis et al. 2015). However, while polyploidy is believed to have played important roles in the evolution of plant and animal lineages, the other major multicellular eukaryotic kingdom—the fungi—poses a puzzle. Although not as well documented as in other systems, polyploidy is observed in many fungi, yet there is little evidence that polyploidy has played a major role in the kingdom's deep evolutionary history. Does this imply that ancient polyploidy (paleopolyploidy) events were not important in the evolution of fungi or merely that we have not discovered traces of these events? Here, we briefly review the role that polyploidy has played in the evolution of nonfungal eukaryotic lineages. We then describe the existing literature on polyploidy in fungi, which underscores the lack of known ancient fungal polyploids. We look to existing data to determine whether a clear pattern of previously unrecognized ancient fungal polyploidization events is present but find

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them insufficient. Therefore, we conclude by outlining ways that the community can move forward to address this important biological question.

Polyploidy Is an Important Evolutionary Force in Eukaryotes

Polyploidy in plants is well recognized, with investigations over many decades identifying a large number of polyploid plant species (Stebbins 1950; Thompson and Lumaret 1992; Wood et al. 2009). Indeed, it is now estimated that 15% of angiosperm and 31% of fern speciation events are associated with an increase in ploidy (Wood et al. 2009). Polyploidy is particularly common among plants that have been domesticated by humans (Renny-Byfield and Wendel 2014), suggesting that our ancestors took advantage of the heterosis (hybrid vigor) exhibited by some polyploids as well as the increased cell size that frequently contributes to the larger organs (hence larger seeds and fruits) found in polyploid plants. In addition, the ability to create artificial polyploids (synthetic polyploids) has put plants at the vanguard of our understanding of the molecular events that result from polyploidy (Skalická et al. 2003; Eilam et al. 2008; Szadkowski et al. 2010; Renny-Byfield and Wendel 2014).

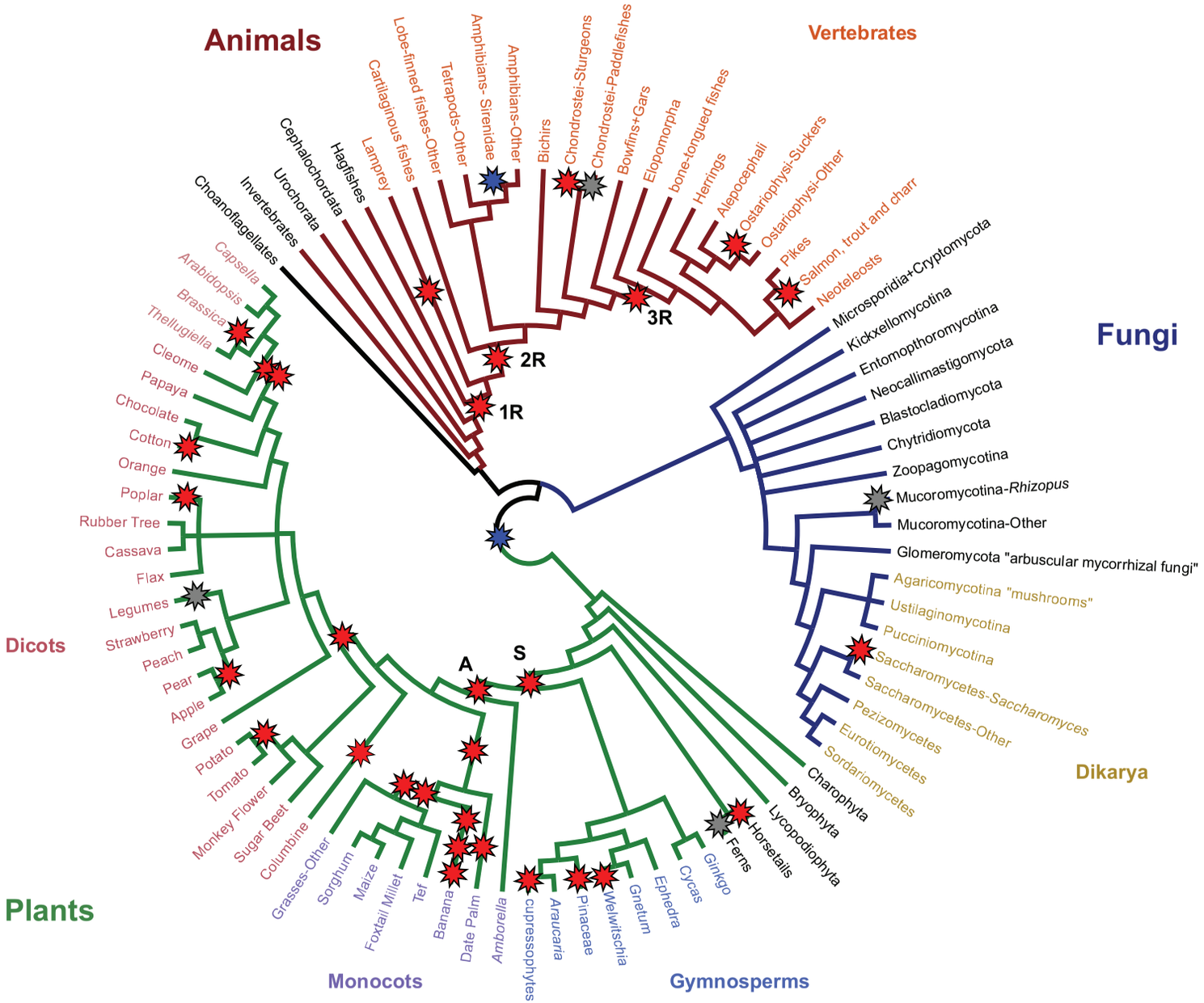
Polyploidy has also been documented in numerous eukaryotic taxa outside of plants. The amphibians (Otto and Whitton 2000; Mable et al. 2011) and fishes (Otto and Whitton 2000; Taylor et al. 2003; Mable et al. 2011) are notable for containing many successful polyploid lineages. Furthermore, polyploidy has been documented in birds (Otto and Whitton 2000), two species of mammal (Gallardo et al. 1999; Mares et al. 2000), crustaceans (Salemaa 1984; Weider 1987; Dufresne and Hebert 1997; Otto and Whitton 2000), insects (Lokki and Saura 1980; Otto and Whitton 2000), and mollusks (Piferrer et al. 2009). Polyploidy can be induced in animals, with synthetic polyploids in fish and shellfish being created for increased food production (Piferrer et al. 2009). In addition, the Chromalveolata contains several polyploid lineages (Green and Dick 1972; Coyer et al. 2006; Albertin and Marullo 2012), and there is evidence in the well-studied fungus-like Oomycota crop pathogen genus *Phytophthora* for recent (Tooley and Therrien 1987; Sansome et al. 1991; Ioos et al. 2006) and perhaps ancient (Martens and Van de Peer 2010; but see van Hooff et al. 2014) polyploidizations. Moreover, polyploidy appears to be commonplace in diatoms (Koester et al. 2010), there are two ancestral polyploidizations in the excavate *Giardia lamblia* (Sun et al. 2010), and the ciliate *Paramecium* exhibits polyploidy (Aury et al. 2006). Therefore, far from being a phenomenon restricted to just a few lineages with unusual biology, polyploidy seems to have occurred repeatedly across a diverse range of eukaryotic taxa.

Ancient Eukaryote Polyploidy

Examples where polyploidy preceded major historical radiations of taxa are key pieces of evidence supporting arguments that widespread polyploidy is observed because it provides a rich source of redundant genetic material to fuel rapid adaptive evolution (Ohno 1970; Selmecki et al. 2015). The best evidence for polyploidy-associated radiations comes from well-characterized ancient polyploidy (paleopolyploidy) events in a number of species-rich plant and vertebrate lineages, where the polyploidy events range from a few to hundreds of millions of years in age. Correlations between these ancient polyploidy events and higher diversification rates in the lineages they produce—such as in certain angiosperm lineages (Soltis et al. 2009) and cyprinine fishes (Zhan et al. 2014)—have further strengthened the case for an association between polyploidy and diversification.

In plants, two major paleopolyploidization events are associated with evolutionary diversifications (fig. 1): one in the common ancestor of all seed plants at around 350 Ma and the other in the common ancestor of all angiosperms at around 235 Ma (Jiao et al. 2011). In addition, numerous paleopolyploidy events in angiosperm taxa subsequent to these two very ancient polyploidizations have been documented (Bowers et al. 2003; Tang et al. 2010; Wendel 2015). In particular, stressful environments appear to increase the incidence of polyploidy in plants, with some of the most successful plant lineages originating with ancient polyploidizations at the time of the Cretaceous-Paleogene boundary (Vanneste et al. 2014*a*, 2014*b*; Soltis et al. 2015). Vertebrates also feature two major ancient polyploidization events: one present at the base of all vertebrates and a second shared by the jawed vertebrates (gnathostomes; fig. 1). These ancient vertebrate polyploidizations occurred at around 600 and 450 Ma, respectively (Vandepoele et al. 2004; Dehal and Boore 2005; Blomme et al. 2006; Nakatani et al. 2007), and are proposed to have driven evolutionary innovation in these groups (Ohno 1970). In addition, a third polyploidization event is thought to have occurred in the common ancestor of all teleost fishes at approximately 320 Ma (Taylor et al. 2003; Vandepoele et al. 2004; Nelson 2006). In sharp contrast, however, only two paleopolyploidy events are known in fungi, and both are relatively shallow events in the fungal tree of life (fig. 1).

Importantly, however, there is not universal agreement that polyploidy is a driver of diversification, with some studies finding no relationship or even a negative relationship between polyploidy and diversification (Santini et al. 2009; Mayrose et al. 2011; Zhan et al. 2014). Moreover, experimental evolution studies comparing populations of different ploidy levels find that the original ploidy in a given species is generally favored (Gerstein and Otto 2009), although most addressed autopolyploids, not allopolyploids. Finally, poly-


Animals
Vertebrates
Fungi
Dikarya
Plants
Dicots
Monocots
Gymnosperms
Lobe-finned fishes-Other
Amphibians- Sirenidae
Amphibians-Other
Bichirs
Chondrostei-Sturgeons
Chondrostei-Paddlefishes
Bowfins+Gars
Elopomorpha
bone-tongued fishes
Herrings
Alepocephali
Ostariophysi-Suckers
Ostariophysi-Other
Pikes
Salmon, trout and charr
Neoteleosts
Cartilaginous fishes
Tetrapods-Other
Lamprey
Hagfishes
Cephalochordata
Urochorata
Invertebrates
Choanoflagellates
3R
2R
1R
A
S
Microsporidia+Cryptomycota
Kickxellomycotina
Entomopthoromycotina
Neocallimastigomycota
Blastocladiomycota
Chytridiomycota
Zoopagomycotina
Mucoromycotina-Rhizopus
Mucoromycotina-Other
Glomeromycota "arbuscular mycorrhizal fungi"
Agaricomycotina "mushrooms"
Ustilaginomycotina
Pucciniomycotina
Saccharomycetes-Saccharomyces
Saccharomycetes-Other
Pezizomycetes
Eurotiomycetes
Sordariomycetes
Charophyta
Bryophyta
Lycopodiophyta
Horsetails
Ferns
Ginkgo
Cycas
Ephedra
Gnetum
Welwitschia
Pinaceae
Araucaria
cupressophytes
Amborella
Date Palm
Banana
Tef
Foxtail Millet
Maize
Sorghum
Grasses-Other
Columbine
Sugar Beet
Monkey Flower
Tomato
Potato
Grape
Apple
Pear
Peach
Strawberry
Legumes
Flax
Cassava
Rubber Tree
Poplar
Orange
Cotton
Chocolate
Papaya
Cleome
Thellugiella
Brassica
Arabidopsis
Capsella

ploidy in plants can be explained by a simple ratchet model of increasing chromosome sets that does not require arguments for selective pressure (Meyers et al. 2006). Therefore, whether polyploidy plays a necessary and sufficient role in evolutionary diversification remains unclear. The other major eukaryote lineage—the fungi—can potentially provide additional data to inform this debate, making a better understanding of the role of polyploidy in fungal evolutionary history important not just for understanding the fungi but also for understanding patterns of diversification more broadly.

Lack of Ancient Polyploidy Events in Fungi

Fungi are a diverse group of organisms that are a sister taxa to the animals (Metazoa) and choanoflagellates (Lang et al. 2002; James et al. 2006). They comprise a huge number of species, estimated to be 1.5 million (Hawksworth 1991; Deacon 2006), with the vast majority yet to be described (Blackwell 2011). While morphologically quite simple apart from their reproductive structures, fungi exhibit enormous diversity in life-history traits, occupying an astonishing range of ecological niches and habitats (Deacon 2006). They also have distinctive reproductive strategies. For many fungi, the haploid phase dominates their life cycle, and many fungi have a dikaryotic growth phase that is characterized by the presence of two different haploid nuclei types (from a mating) in the same cell that have not been fused into a diploid nucleus. Moreover, many fungi appear to lack a sexual stage completely, and the majority of these asexual species live as haploids. As a consequence, the majority of fungi live in a haploid nuclear state for most of their life cycle (Deacon 2006).

These unique features mean that the use of the terms "polyploid" and "hybrid," which are well defined in the plant and animal literature, are less clear for fungi. Polyploidy can be defined as "the heritable condition of possessing more than two complete sets of chromosomes" (Comai 2005, p. 836). However, because fungi are typically haploid for the major part of their life cycle, a genome doubling in a fungus—thus creating a diploid—would not be considered a polyploid under this traditional definition, despite the ploidy level differing from the parents. The fungal literature often refers to these cases as hybrids (offspring of mating between two different species; Mallet 2007) rather than polyploids. To accommodate fungi into the traditional conceptual framework of polyploidy, we adopt the following terminology (fig. A1; figs. A1–A3 available online): hybrids are offspring of two different species (that may or may not have altered numbers of chromosome sets). If a hybrid has a heritable increase in the number of chromosome sets relative to the parents, it is an allopolyploid, including allodiploids (where two haploid species have merged to produce a diploid). Following the same logic, we describe intraspecific doublings (or more) of chromosome sets as autopolyploids, including examples where a haploid species doubles to a diploid.

Polyploidy has not been well studied in the fungi, despite the most widely used model fungal species—baker's yeast (*Saccharomyces cerevisiae*)—having a paleopolyploidy event in its evolutionary history (Albertin and Marullo 2012). To provide an overview of the current state of fungal polyploidy, we compiled a list that encompasses all 31 different genera with reported fungal polyploids (app. B; apps. A, B available online). Although known fungal polyploids are fewer compared with plants and animals, they are phylogenetically widespread (fig. 2; app. B). Interestingly, in parallel with plants where a large number of domesticated species are polyploids, several fungi that have been domesticated or are associated with humans are also polyploids (for a more detailed discussion, see app. A). However, because the vast majority of fungal species have not been tested to determine whether they are polyploid, it is likely that the polyploids encompassed by these 31 genera (app. B) are a dramatic underestimate of the true number.

When we consider the approximate time of origin of these documented fungal polyploids, most have resulted from relatively recent events, with only two known examples of ancient fungal polyploidy (fig. 3). The first is the iconic example of yeasts of the genus *Saccharomyces* (Albertin and Marullo 2012). This yeast paleopolyploidization event has been dated to about 100 Ma (Wolfe and Shields 1997) and most likely results from an ancient allopolyploidy event (Marcet-Houben and Gabaldón 2015). The second example of ancient polyploidization occurs in the zygomycete genus *Rhizopus*, although whether this was an auto- or allopolyploidy event is unknown, and the origin has not been dated (Ma et al. 2009; Shelest and Voigt 2014). A striking conclusion from these observations is that despite infrequent but widespread observations of recent polyploidy events, there is no evidence of polyploidy events having shaped the deep evolutionary past for the vast majority of fungal species. This is in sharp contrast to species-rich plant and animal lineages, many of which have a least one polyploidy event in their

Figure 1: Ancient polyploidization events (paleopolyploidizations) involving genera or higher taxonomic levels are indicated with starbursts on a cladogram of plant, fungal, and animal relationships. Red starbursts indicate well-characterized paleopolyploidy events, gray starbursts indicate ancient polyploidizations with uncertainty in taxonomic distribution due to limited numbers of genome sequences in the group, and blue starbursts indicate proposed ancient polyploidies that are not confirmed. For further information, see appendix A, available online. Seed plant (S) and angiosperm (A) ancient polyploidies are indicated. Vertebrate ancient whole-genome duplications known as 1R and 2R are labeled as such, with the fish-specific teleost paleopolyploidization event labeled 3R. Other eukaryotic lineages have been omitted for clarity.

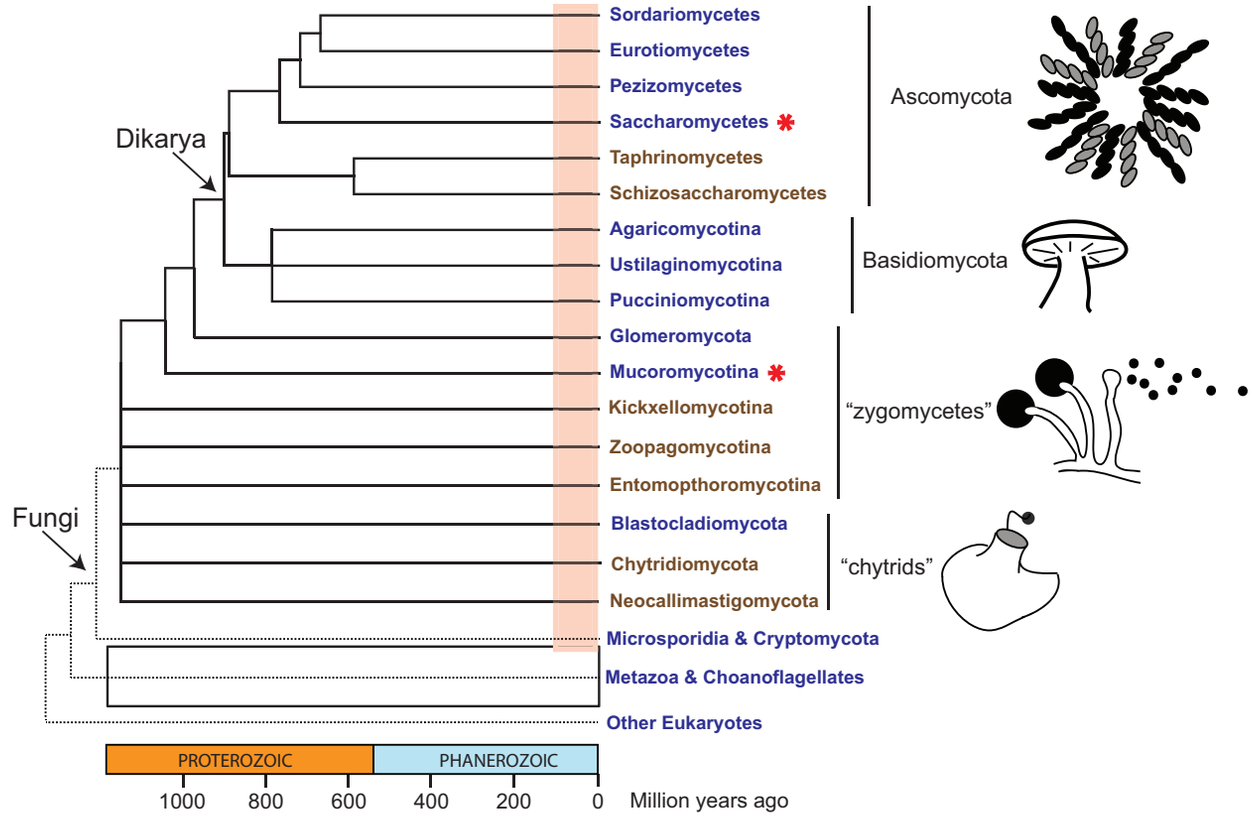


Figure 2: Major extant fungal lineages are depicted (adapted from Blair 2009). Organization of the four major fungal groups (Ascomycota, Basidiomycota, zygomycetes, and chytrids) is indicated alongside representative depictions of the four groups: asci from *Neurospora* (Ascomycota), a mushroom (Basidiomycota), a sugar/pin mold (zygomycetes), and a chytrid sporangium with a zoospore. The basal fungal lineages remain poorly resolved (Lang et al. 2002; Ebersberger et al. 2011) and are shown as a polytomy. The nearest relatives of fungi, including the most basal groups (Microsporidia and Cryptomycota), are the Metazoa (animals) and Choanoflagellates (James et al. 2013; Burki 2014). Blue indicates polyploid lineages; brown indicates those without. Vertical red shading indicates the period in the fungal phylogeny during which polyploidy events are known to have occurred. Red asterisks indicate lineages with the oldest known fungal polyploidy events, *Saccharomyces* yeasts (Saccharomycetes) and *Rhizopus* (Mucoromycotina). Solid branches are proportional to time (indicated by the timescale); dotted branches are not.

evolutionary past and some of which have many (fig. 1). This is not simply a result of the fungi being a small lineage, since the estimated 1.5 million fungal species (Hawksworth 1991) exceeds the estimated 450,000 angiosperm species (Pimm and Joppa 2015). Therefore, it appears that the lack of ancient fungal polyploidy events is a phenomenon that needs explanation.

Where Are the Missing Fungal Polyploids?

This dissonant picture of polyploidy in fungi—where most polyploids are recently derived and none appear older than 100 million years, despite fungi having originated approximately 800 million years ago (Taylor and Berbee 2006; fig. 2)—has three possible explanations. First, ancient polyploidy events were extremely rare and are thus largely absent from the evolutionary history of fungi. Second, polyploidy events occurred frequently, but most created evolutionary dead ends that did not lead to extant lineages. Third, ancient polyploidy events made important contributions to fungal evolution but have largely gone unrecognized. We address each of these possible explanations.

Ancient Fungal Polyploidy Events Were Rare, and Few Extant Fungi Have Ancient Polyploid Origins

We suggest that this option is unlikely: recent polyploidy events have been found in a diverse range of modern fungal lineages (fig. 2), and there is nothing peculiar to fungi to suggest that polyploidy should have been less common in the past. Synthetic fungal polyploids have been made artificially (Ishitani et al. 1956; Holliday 1961; Maniotis 1980), with some synthetic polyploids generated from fungal lineages that have no known natural polyploids (Kostoff 1946; San-

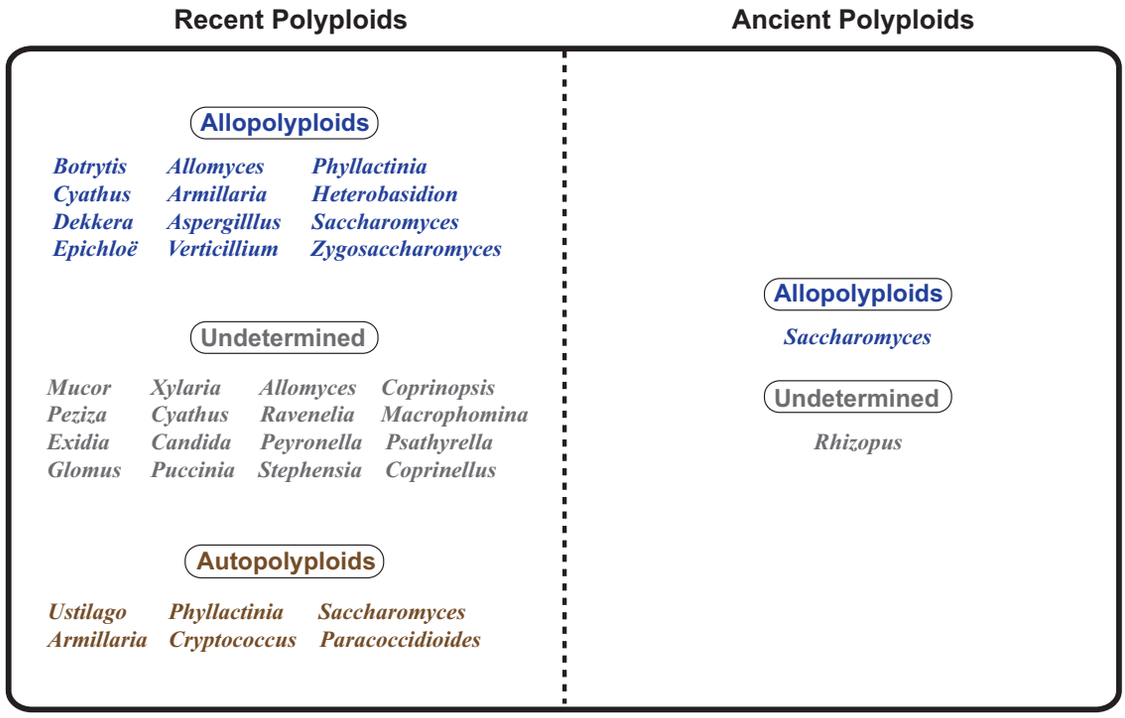


Figure 3: Fungal polyploids are listed by genus as either recent (neo-) or ancient (paleo-) polyploids. Auto- or allo polyploidy is indicated where known. Only two ancient fungal polyploidy events are known. For further details, see appendix B, available online.

some 1946; Day 1972*a*, 1972*b*; Cummins and Day 1977). These observations suggest that the ability to form viable polyploids is common in fungal lineages and is not restricted to a few lineages with unusual biology. While chromosome-based sex determination may be a major barrier to polyploidization in animals (Otto and Whitton 2000), this system does not occur in fungi. Therefore, no significant barriers appear to prevent polyploidy in fungi. However, many fungi live as multinucleated forms, particularly dikaryons (two genomically different nuclei within the same cell; see fig. A1) for much of their life cycle. This ability may circumvent the need for polyploidy to gain the benefits of combining two separate genomes. Consequently, it remains conceivable that ancient fungal lineages favored a multinuclear hybrid state without forming true polyploids. This phenomenon would produce short-term advantages but would not produce the long-term genetic redundancy through which polyploidy is proposed to drive diversification. Nevertheless, recent fungal polyploids are still observed, including in groups with multiple independent events, suggesting that polyploidy can offer selective advantages over—or instead of—dikaryotic hybrids.

Ancient Fungal Polyploids Were Evolutionary Dead Ends

It has been argued that polyploidy does not necessarily provide benefits and instead is often maladaptive, characterized by initial instability, numerous genomic challenges, and higher extinction rates (McClintock 1984; Comai 2005; Mayrose et al. 2011). This viewpoint suggests that the reason ancient fungal polyploids are not observed is because they were evolutionary dead ends. However, the data used to support this contention come from plant and animal systems, where ancient polyploidy is well known and has been linked to evolutionary radiations. A more compelling argument comes from one of the best-studied fungal genera, *Epichloë*. All of the numerous polyploid species from this group characterized to date are asexual (Moon et al. 2007; Leuchtmann et al. 2014), and asexual lineages are generally considered to be evolutionary dead ends (Muller 1964; Maynard 1978). However, other fungal polyploids, such as the *Saccharomyces* yeasts, are sexual, suggesting that, as with plants and animals, fungal polyploidy can produce persistent sexual lineages. Fungi have unique properties that may reduce the potential selective advantages of polyploidy. In particular, the high level of chromosomal variability, in terms of both number and structure, that is exhibited by fungi (Covert 1998; Ma et al. 2010; Croll and McDonald 2012; Raffaele and Kamoun 2012), uni/parasexuality (Heitman 2010), and aneuploidy (Tolmsoff 1983) may provide similar benefits to polyploidy without the potentially deleterious issues that arise from genome merger. Therefore it remains possible that polyploidy did not provide the selective advantages for fungi

that it did for other lineages, but this would require that extant fungal diversity has been driven by something other than the genetic redundancy created by polyploidy.

Ancient Fungal Polyploidy Events Made Important Contributions to Fungal Evolution but Remain Largely Unrecognized

There are two primary reasons why polyploidy, particularly polyploidy events in the deep evolutionary past, might have gone unrecognized in the fungi. First, karyotypes have long been a primary form of genomic characterization in plants and animals, and chromosome counts have commonly been employed to infer polyploidy events (Maniotis 1980; Mayrose et al. 2010). However, it is challenging to observe fungal chromosomes with conventional microscopy due to their small size and relatively low level of condensation at metaphase, as well as the continued presence of the nuclear membrane through most of mitosis in some lineages (Deacon 2006). At present, the favored karyotyping method for fungi is pulsed field gel electrophoresis, which is a relatively recent technique, is technically difficult to perform and cannot easily produce unambiguous chromosome counts. Consequently, karyotyping has not been widely performed for fungi, and chromosome counts are not readily available as a relatively simple avenue for identifying polyploidy. Second, paleopolyploidy events deep in the tree of life are difficult to detect, requiring multiple supporting lines of evidence and sophisticated genome-scale analyses (e.g., Ma et al. 2009; Marcet-Houben et al. 2009; Marcet-Houben and Gabaldón 2015). Such analyses have typically been performed only where there is prior evidence for ancient polyploidy, such as when karyotypes indicate a change in chromosome complement consistent with polyploidy or when numerous syntenic blocks are observed during genome sequencing. In addition, the genomic signatures of ancient polyploidy may be more difficult to detect in fungi, which exhibit rapid and extensive genome restructuring over short evolutionary time frames (Croll and McDonald 2012; Raffaele and Kamoun 2012). The apparent lack of ancient polyploidy in fungi may therefore result from a deficit of information used to infer polyploidy. Given this and the prevalence of paleopolyploidy in plant and animal lineages, we propose that there are a significant number of unrecognized paleopolyploidy events in the deep evolutionary history of fungi.

Can Existing Data Inform the Presence of Ancient Fungal Polyploids?

We wondered whether there are strong signals of fungal polyploidy in existing data that have simply been overlooked. To address this, we turned to chromosome count data because large changes in chromosome number may be indicative of polyploidy events. While difficult to obtain for fungi, chromosome counts are nevertheless available for a number of species. We found published chromosome counts for 25 fungal species and modeled the evolution of chromosome number in these species using a probabilistic framework to identify potential polyploidization events (described in app. A). However, while polyploidy was identified for the *Saccharomyces* yeasts as expected, the low proportion of fungi that have chromosome count data available means that the analysis has statistical power to detect polyploidy in only a very small subset of fungal taxa. Therefore, the primary conclusion from this analysis is that existing chromosome count data are too limited to test the hypothesis that there are unrecognized polyploidy events in the deep evolutionary history of the fungi.

Moving Forward

To solve the puzzle of whether there are unrecognized polyploidy events deep in the evolutionary history of the fungi, more data are clearly needed. Encouragingly, the evidence required to address the extent of ancient polyploidy in fungi is increasingly becoming available, aided by the relatively small genome sizes of fungi. Two complementary types of data will help investigate the role of polyploidy in fungal evolution: (1) additional chromosome count data and (2) genomic sequence data.

Given the difficulties of observing condensed fungal chromosomes (Kohn 1992), the best source of chromosome counts for fungi still remains pulsed field gel electrophoresis. While obtaining precise chromosome counts from these gels is challenging, estimates remain valuable for detecting large shifts in chromosome number resulting from polyploidy events. Other techniques—such as hybridization-based detection of telomeres (Ijdo et al. 1991; Garrido et al. 2012), fluorescent detection of the centromere-specific histone variant (Henikoff et al. 2001; Cleveland et al. 2003; Jin et al. 2008; Shibata et al. 2013; Tek et al. 2014), and optical mapping (Schwartz et al. 1993)—could also be employed. However, as DNA sequencing technologies and genome assembly algorithms improve, chromosome-level assemblies are soon likely to provide the most accessible means of obtaining chromosome counts (Marie-Nelly et al. 2014; Faino et al. 2015). Broader coverage of chromosome counts across the fungal phylogeny will then allow chromosome modeling methods, such as the one we employed above, to generate more robust predictions of polyploidy.

The most powerful way to detect polyploidy and, in particular, ancient polyploidy is through genome sequence analysis. A number of sophisticated analyses are now available for inferring polyploidy events from genomic data. These include synteny-based methods that have revealed ancient polyploidization events in *Saccharomyces* yeasts and *Rhizopus* (Kellis et al. 2004; Ma et al. 2009). Synteny comparisons

identify homologous regions within and between genomes, with patterns of conserved gene order for suites of gene duplicates across a genome providing evidence for genome duplication through polyploidy. Such approaches have been widely used in eukaryotes, particularly in plants, but rapid genome rearrangements (Fischer et al. 2006; Ma et al. 2010; Stukenbrock 2013) reduce the power of these approaches for fungi, making the identification of paleopolyploids more challenging than for plants or animals at comparable timescales.

Another widely used method employs synonymous substitutions, which are unaffected by the strong pressures on nonsynonymous substitutions. Polyploidization results in the creation of multitudinous paralogous gene pairs, and for each pair of paralogs, a per-site synonymous divergence can be calculated (K_s). The mass origin of paralogs at the same time resulting from polyploidization will create a peak in the distribution of K_s values, and the value of K_s on which a peak is centered serves as a proxy for time (Lynch and Conery 2000, 2003). However, the ability of a K_s-based approach to detect paleopolyploidy decreases in power as the age of the polyploidy event increases and is subject to effects of saturation and stochasticity, leading to decreased signal and artificial indications of paleopolyploidy (Cui et al. 2006; Vanneste et al. 2013).

Phylogenomic approaches encompass a more recently developed suite of methods that also use protein-coding genes to provide evidence for ancient polyploidy. The rationale is that duplications observed in a gene tree are bounded by the lineages that diverged before and after them. Using gene tree/species tree comparisons, these duplications can be dated (Huerta-Cepas et al. 2007; Huerta-Cepas and Gabaldón 2011). A peak in the number of observed gene duplications on a given branch of the species tree provides strong support for a whole-genome duplication event at that point. A particular strength of phylogenomic approaches is that the origin (auto- or allopolyploidy) can increasingly be detected. For instance, a recent phylogenomic analysis of the ancient polyploidization event in the *Saccharomyces* lineage provided evidence that this ancestral event likely resulted from an ancient allo- (rather than auto-) polyploidization event (Marcet-Houben and Gabaldón 2015). Specifically, support for this scenario came from the surprising finding that paralogs (ohnologs) derived from the whole-genome duplication event had already diverged before the chromosome doubling event occurred, indicating that they were present as two distinct lineages at the time of the polyploidization. As the number and taxonomic diversity of fully sequenced genomes increases, so too does the power of phylogenomic approaches to detect and date gene duplicates. Therefore, this approach will increasingly be useful for identifying ancestral polyploidization events and disentangling their origins.

Finally, another potential avenue for identifying fungal paleopolyploidy may be modeled after vertebrate research. The oldest vertebrate genome duplications were supported by patterns of paralogy in *HOX* gene clusters (Ohno 1999; Popovici et al. 2001; Blomme et al. 2006), which are associated with the evolution of body plans and occur as a single cluster in invertebrates but as four clusters on different chromosomes in humans (Garcia-Fernandez 2005; Nakatani et al. 2007). If analogous conserved gene clusters are found in fungi, they may help to identify paleopolyploidy events >100 million years ago.

Regardless of which methods are used, a broader and deeper sampling of fungal genome diversity will be required to determine whether polyploidy events deep in the evolutionary history of fungi really are more widespread than is currently observed. The generation and analysis of fungal chromosome count data will aid in this by providing initial predictions of ancient fungal polyploidy events to target subsequent effort using genomic approaches that are not trivial to perform.

Conclusions

The role that ancient polyploidy has played in the evolutionary history of the fungi remains unclear. Polytomies in the fungal tree of life (figs. 1, 2) that are difficult to resolve (Lang et al. 2002; Ebersberger et al. 2011) and the large number of species-rich lineages (Deacon 2006; Stajich et al. 2009) are both suggestive of rapid historical radiations. If polyploidy is a critical contributing factor for evolutionary radiations, we would expect to find evidence for polyploidy at the base of these lineages. Conversely, a lack of polyploidy events underpinning significant fungal radiation episodes would suggest that eukaryotic evolutionary diversification is not necessarily linked to polyploidy. Thus, determining the extent of ancient fungal polyploidy and its role in fungal evolutionary history is an important component of the debate as to whether polyploidy really is a major driver of species diversifications across the eukaryotes as a whole, as well as to obtain a better understanding of the evolutionary history of this important group of organisms.

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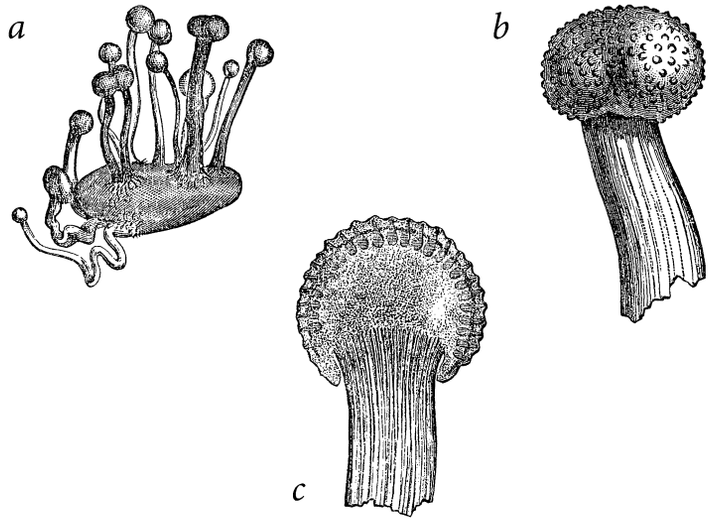

"*a*, Ergot of Wheat producing the small Fungus, *Claviceps purpurea*, Tul.; *b*, one of the heads magnified. *c*, section through a head, to show the cavities containing the spores." From "On Ergot" by William Carruthers, *The American Naturalist* 1875, 9:450–465.