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Review

Polyploidy in fungi: evolution after whole-genome duplication

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Polyploidy is a major evolutionary process in eukaryotes—particularly in plants and, to a less extent, in animals, wherein several past and recent whole-genome duplication events have been described. Surprisingly, the incidence of polyploidy in other eukaryote kingdoms, particularly within fungi, remained largely disregarded by the scientific community working on the evolutionary consequences of polyploidy. Recent studies have significantly increased our knowledge of the occurrence and evolutionary significance of fungal polyploidy. The ecological, structural and functional consequences of polyploidy in fungi are reviewed here and compared with the knowledge acquired with conventional plant and animal models. In particular, the genus *Saccharomyces* emerges as a relevant model for polyploid studies, in addition to plant and animal models.

Keywords: polyploid; palaeopolyploid; whole-genome duplication; hybridization; reticulate evolution

1. INTRODUCTION

Polyploidy (definitions are given in box 1) has long been considered as a prominent process shaping eukaryotes evolution [1,2]. Several well-described natural polyploid organisms are known, such as oilseed rape (that combines both cabbage and turnip mustard [3]), cotton, wheat, goldfish or grey treefrog (for a review, see Otto & Whitton [2]). In addition to these recent polyploids, many ancient polyploidization events (also called palaeopolyploidization) were described in the evolutionary history of several taxa such as in angiosperms or vertebrates [4,5]. Although past and recent polyploidization events occurred repeatedly in the animal kingdom [2], it is particularly prominent in plants and especially in angiosperms. As pointed out by Soltis *et al.* [6], the actual question is no longer to know how many flowering plants are polyploids but how many polyploidization events occurred within each angiosperm lineage. Indeed, most of the knowledge regarding polyploid occurrence and evolution was obtained using plant models, and to a lesser extent using animals [2]. Surprisingly, the incidence of polyploidy in other large eukaryote kingdoms, such as the fungi, remains largely unknown despite numerous data collected for years. Polyploidy in fungi is usually evoked (and reduced to) the well-described whole-genome duplication (WGD) that occurred in yeast lineage about 100 Ma [7].

There are several reasons why the works dealing with fungal polyploids remain disregarded by non-specialists of mycology. Firstly, polyploidy in fungi has long been viewed as rare or absent [8], essentially because most reported haploid chromosome numbers were low, i.e. in the range of 4–8 [9]. Secondly, much of the available data were collected before the 1980s [10] and are poorly accessed now, whereas later works were published in very general fungal books so that the 'polyploid section' remains confidential for non-mycologists [11]. Thirdly, recent data were obtained, especially on polyploid yeasts of the *Saccharomyces* genus, but were hidden by the huge amount of publications dealing with non-polyploid yeasts. In fact, a few strains of *Saccharomyces cerevisiae* progressively came to dominate basic research the past four decades. These laboratory strains (S288C, W303, FL100, etc.) were initially selected for their simplified genetic manipulation, and were de facto chosen among diploid strains and/or their haploid derivatives. As a consequence, these laboratory haploid/diploid strains are now massively represented in yeast works. While the proportion of publications dedicated to polyploid *Saccharomyces* sp. represented about 30 per cent before the 1970s, it falls under 10 per cent until 2000 (figure 1). The past decade shows a little renewal in the interest in polyploid *Saccharomyces* with 13–15% of the total publications, yet falling within the scope of applied research rather than understanding evolutionary phenomena. For example, polyploid strains are used as models for cancer or cell cycle defects studies [12,13]. In addition, many industrial yeasts used in bakery, brewery, etc., are polyploids [14], so that several biotechnological-orientated works were described recently [15] but are not easily accessed by the scientific community working on the evolutionary consequences of polyploidy.

The aim of this work is thus to summarize the knowledge acquired on polyploid fungi, with a special emphasis on yeasts for which recent data are available. Issues that are

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Box 1. Definitions.

Polyploidy is the state of having three or more sets of chromosomes in contrast to the two sets present in diploids (and one in haploids). The sets of chromosomes may originate from a single species (*autopolyploidy*) or from different ones, generally closely related (*allopolyploidy*). The polyploid status is heritable through the germ line: meiosis in polyploids leads to the formation of gametes having two or more chromosome sets.

Aneuploidy designs the occurrence of one or more extra or missing chromosomes by comparison with the normal haploid/diploid state of the species. When considering polyploids, the aneuploid level is intermediary between polyploid and diploid ones and may result from the diploidization process.

Endopolyploidy, or somatic polyploidy, arises through recurrent cycles of DNA replication without cellular division *via* either endoreduplication or endomitosis processes. Somatic polyploidy is generally associated with cellular differentiation or specific stages of life cycle (cyclic polyploidy) and results in genome content increase in the somatic line, not in the germ line. Thus, it is not heritable through sexual reproduction.

Diploidization is the process by which a polyploid organism returns to a diploid mode of chromosome pairing. Diploidization may involve various mechanisms, including partial or full chromosome losses, genome rearrangement, sequence divergence and deletion allowing the differentiation of the duplicated chromosomes and the apparition of diploid-like behaviour at meiosis. Diploidization leads to *palaeopolyploid* organisms that retain only traces of the past polyploidization event(s) on their genome.

Hybridization: merging of genomes from two different species (interspecific hybridization) or two different individuals of the same species (intraspecific hybridization).

Homoploid speciation is hybrid speciation without a change in chromosome number (without genome doubling).

Reticulate evolution is characterized by occasional hybridization, backcrosses and combination of two species. Reticulate evolution is frequently described in taxa prone to polyploidy.

Homologous: chromosomes or genes derived from a common ancestor.

Homeologous: paralogous chromosomes or genes merged within a single nucleus in allopolyploids. Homeologous genes are also referred to as homeoalleles.

Neopolyploid: newly generated polyploid individuals (also referred as *synthetic polyploid*), generally induced through artificial means (colchicine treatment, etc.).

traditionally studied with plant and animal models are focused on. In particular, the ecological, structural and functional consequences of polyploidy in fungi are addressed.

2. THE OCCURRENCE OF POLYPLOIDY IN FUNGI

The fungal kingdom comprises more than 100 000 described species [16]. The precise taxonomy of fungi is constantly evolving alongside the acquisition of new genomic data, and some fungi-like taxa, such as the Oomycetes lineage, are no longer included among the fungi kingdom (box 2 and figure 2).

A recent phylogenetic classification of the true fungi described 11 phyla (figure 3), four of them having uncertain position (Mucoromycotina, Kickxellomycotina, Zoopagomycotina and Entomophthoromycotina are incertae sedis phyla) [19]. Several species were identified as natural polyploids (figure 3). For example *Rhizopus oryzae*, a human pathogen, was the first fungus from the early lineages of the fungi kingdom whose genome was fully sequenced [20]. Subsequent genomic analysis revealed that its evolutionary history was marked by a WGD followed by diploidization [20]. More recent fungal polyploidization events were identified within the Blastocladiomycota phylum, particularly among aquatic fungi (*Allomyces* sp.) that display polyploid series containing autotriploid, autotetraploid and allotetraploid representatives [21]. The Glomeromycota phylum comprises arbuscular mycorrhizal fungi that are thought to be the oldest group of asexual multicellular organisms. In a recent publication, Pawlowska & Tslor [22] demonstrated the existence of polyploid nucleus within *Glomus etunicatum*, and suggested that genome polyploidization might account for their long-term evolutionary persistence in the absence of sexual reproduction. Natural polyploidization events were also identified within the Basidiomycota phylum where several edible mushrooms and relatives may be polyploids (figure 3). For example, *Cyathus stercoreus*, commonly known as the dung-loving bird's nest, is a tetraploid species (possibly allotetraploid) displaying tetravalent formation at meiosis between its more closely related homeologous chromosomes [23]. Not surprisingly, the largest phylum of fungi, the Ascomycota, contains many polyploids: within both *Pezizomycotina* and *Saccharomycotina* subphyla, several studies suggested the existence of both inter and intraspecific polyploids within the *Phyllactinia*, *Stephensia*, *Xylaria*, *Botrytis* and *Zygosaccharomyces* genus (figure 3 and electronic supplementary material, table S1) [24,25]. However, to date, most of the evidenced polyploid fungi belong to the well-described *Saccharomyces* genus.

(a) Saccharomyces *genus evolution: a polyploid story*

The yeast *S. cerevisiae* has been exploited by humans for millennia to produce alcoholic beverages, including beer [26], wine [27] and spirits, or to leaven bread [28]. Besides its importance for several food industries, *S. cerevisiae* is one of the most intensively studied eukaryote models in molecular and cell biology, and was the first eukaryote whose genome was fully sequenced [29]. The analysis of the genome sequence revealed a WGD in the evolutionary history of the *Saccharomyces* genus [7], as first suggested by Smith [30]. The yeast WGD occurred after the divergence of *Saccharomyces* from *Kluyveromyces* around 100 Ma and was followed by subsequent diploidization, which is defined as the 'process by which a polyploid genome turns into a diploid one' [31]. In addition to this ancient WGD, several studies revealed that an important number of yeasts are recent polyploids [32]. For example, a genetic analysis of different *S. cerevisiae* food-processing strains revealed a noteworthy proportion of autotetraploids (10 of 26 strains) displaying tetrasomic inheritance at meiosis [33]. A polyploid population (possibly autotetraploid) was isolated from pearl millet beer in West Africa [34]. This population displays almost separate sexes, suggesting a shift from usual yeast hermaphroditism to a near-dioecious behaviour [34]. Many *Saccharomyces* strains used for wine-making were also proved to be polyploid such as for Tokaj wine-making in Slovakia and Hungary [35] or

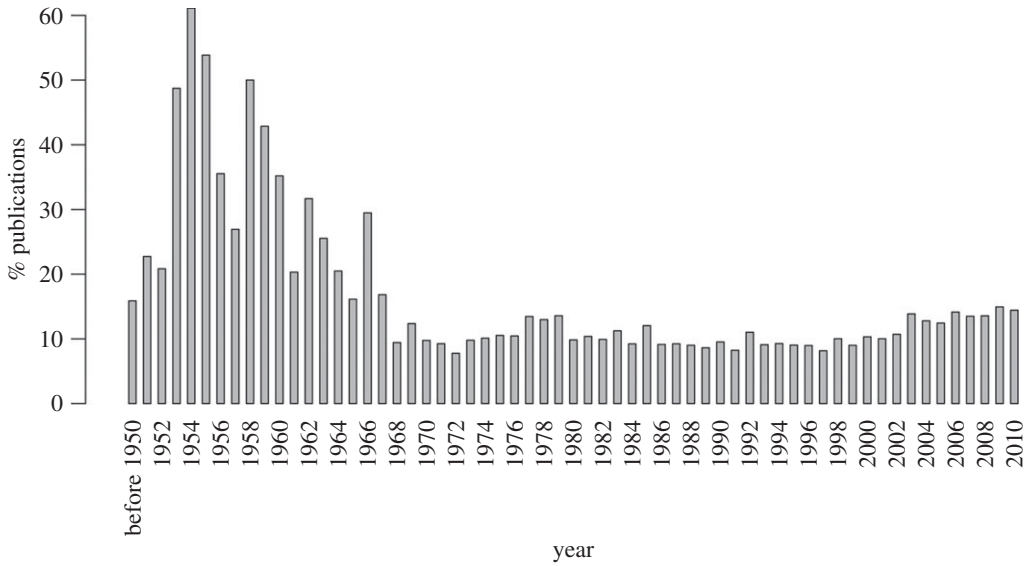

Figure 1. Annual proportion of publications addressing polyploidy within the *Saccharomyces* genus. The annual number of publications addressing either '*Saccharomyces* tetraploid OR polyploid' or '*Saccharomyces* haploid OR diploid -tetraploid -polyploid' was estimated using Google Scholar (release of May 2011).

Box 2. Polyploidy in other eukaryotic taxa.

Besides plants, animals and fungi, other eukaryotic taxa have experienced one or more polyploidization events during their evolutionary history. The oomycetes, which are non-true fungi members, contain several examples of polyploid species, such as within the *Phytophthora* genus (figure 2 and electronic supplementary material, table S4). Some species of brown algae (Fucales, Laminariales and diatoms) contain apparent polyploid genomes. In the Alveolata group, the remarkable species *Paramecium tetraurelia* underwent three successive rounds of WGD [17] and established itself as a major model for palaeopolyploid studies. Thus, far from being restricted to plants and animals, polyploidy is more likely a general process of eukaryotic evolution.

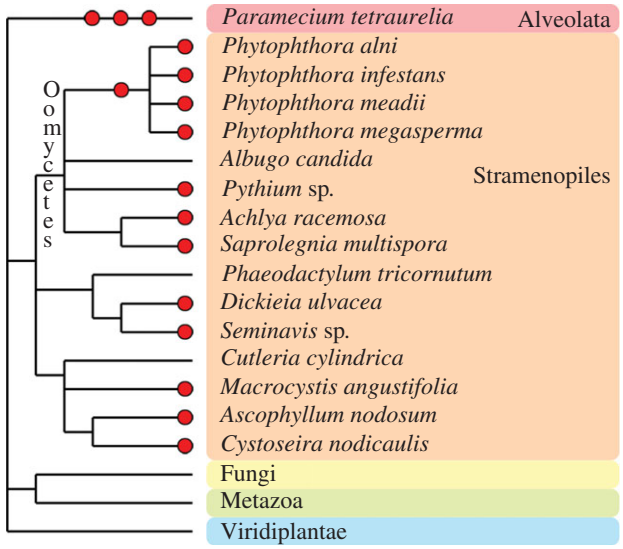

Figure 2. Inferred polyploidy and reticulate evolution in the Chromalveolata eukaryote supergroup. Schematic phylogeny and classification of the Chromalveolata eukaryote supergroup base on the taxonomy database maintained by the UniProt group NEWT [18] (release of May 2011). Branch lengths are not proportional to genetic distances. Red circles indicate suspected polyploidy. Full references are available in electronic supplementary material, table S4.

Spanish sherry-type wines [36]. A well-known example of allopolyploid speciation in yeast is the formation of *Saccharomyces pastorianus*, widely used in brewery to produce lager beer. Its allotetraploid origin was first suggested by Nilsson-Tillgren *et al.* [37], yet *S. pastorianus* progenitors were elucidated later as *S. cerevisiae* [38,39] and an unidentified species close to *Saccharomyces bayanus* [38,40,41]. Recently, Libkind *et al.* [42] established that the *S. bayanus*-like genome donor was actually a new species designed as *Saccharomyces eubayanus*. The identification of many polyploid *Saccharomyces* yeasts associated with different food-processing contexts led to their biotechnological exploitation in applied research [43,44] and hid the occurrence of polyploidy in non-industrial yeasts. However, recent works indicated that polyploidy is not restricted to food process, with the identification of polyploid series (ha-, di-, tri- and tetraploids) from soil isolates in Israel and opportunist *Saccharomyces* polyploids from clinical isolates [45,46].

(b) *Polyploidy and hybridization*

Polyploidization and hybridization are closely interrelated processes: allopolyploidy necessarily arises through interspecific hybridization associated with genome doubling. In addition, although autopolyploidy may arise without intraspecific hybridization (i.e. only through genome doubling), many autopolyploid species display higher heterozygosity levels than their diploid counterparts as in plants or yeasts [33,47], suggesting a hybrid origin. Evolution through hybridization, with or without genome doubling, is referred as reticulate evolution or reticulation [48] and may be the first step towards polyploidy. Until the 1990s, hybridization in fungi was considered to be rare [49] but several fungal hybrids have been described since then (electronic supplementary material, table S2).

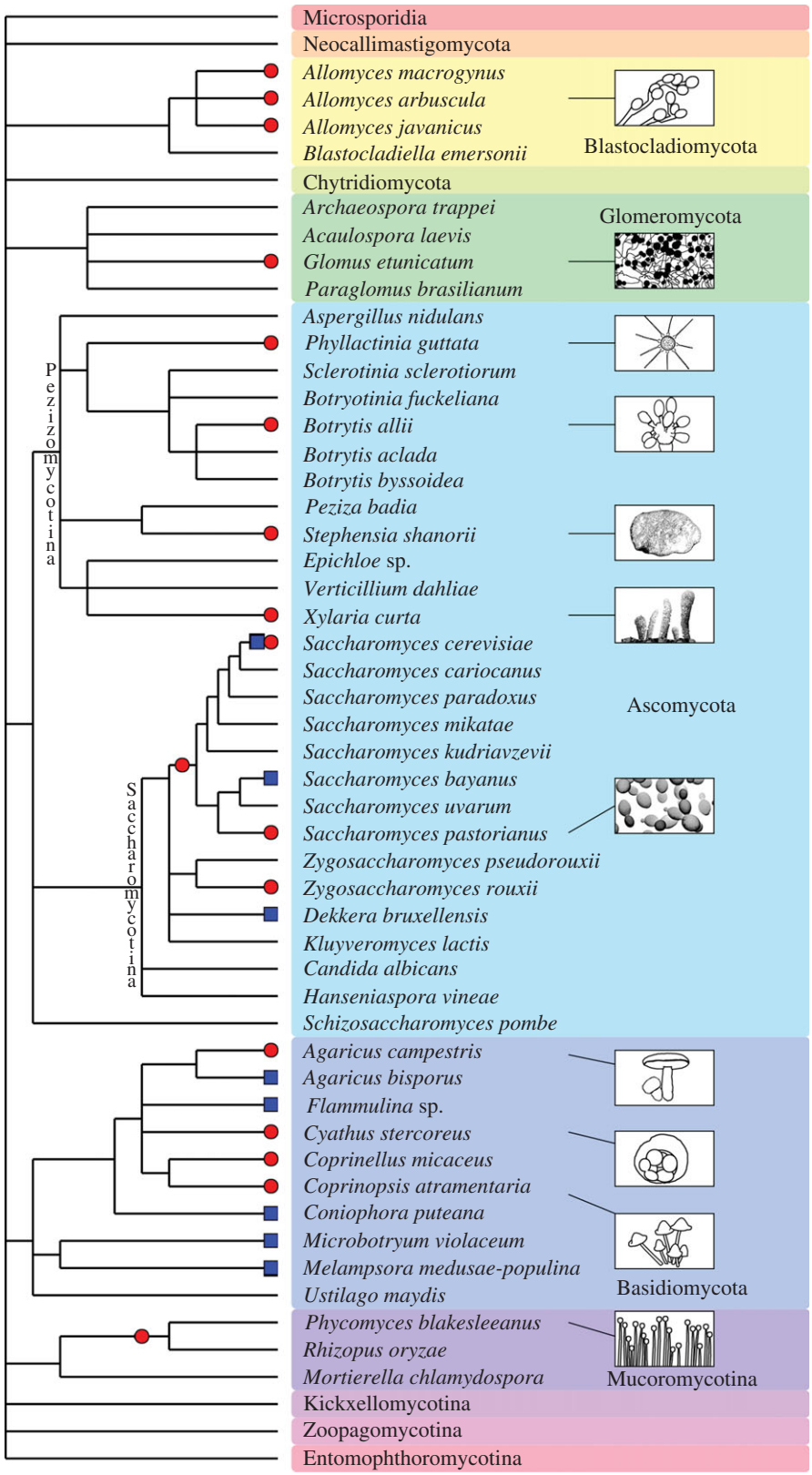

Figure 3. Inferred polyploidy and reticulate evolution in fungi. Schematic phylogeny and classification of the fungi, based on Hibbet *et al.* [19]. Red circles indicate suspected polyploidy, blue squares indicate lineages with individuals having hybrid origin (reticulate evolution). Branch lengths are not proportional to genetic distances. Full references are available in electronic supplementary material, tables S1 and S2.

For example, interspecific fungal hybrids were described such as in the phytopathogen species *Verticillium dahliae* [50] and *Melampsora* × *columbiana* [51,52], the cultivated mushroom *Agaricus bisporus* [53] and other edible mushrooms from the *Flammulina* genus [54]. The *Saccharomyces* genus is particularly prone to interspecific hybridization: natural *S. cerevisiae* × *Saccharomyces kudriavzevii* and *S. cerevisiae* × *S. bayanus* hybrids have been repeatedly reported and may be much more frequent than initially thought [55–57]. A striking example of the

Saccharomyces genus ability to mate is the strain CID1, used for cider production (a fermented beverage from apple juice) in France, which is a 'triple' hybrid having at least pieces of *S. cerevisiae*, *S. kudriavzevii* and *S. bayanus* genomes [58]. *Saccharomyces bayanus* is now established to be a complex hybrid species, with genome contributions from *S. uvarum*, *S. eubayanus* and to a less extent *S. cerevisiae* [42,59], explaining the difficulties and incongruities encountered for the identification of *S. bayanus* strains. The spoilage yeast *Dekkera bruxellensis*, which is responsible for the undesirable 'Brett character' in wine, has a complex and dynamic genome that originated through interspecific hybridization, aneuploidization and polyploidization [60]. An inter-family hybrid was also described between *Hanseniaspora vineae* and *S. cerevisiae* [61]. In addition to these natural hybrids, there are several reports of successful construction of interspecific and inter-genera fungal hybrids in the laboratory [62–64], illustrating the genome plasticity of fungi regarding genome merging.

Hybridization may be followed by backcrosses with one parental species, allowing the recovering of a parental-like species bearing a few introgressed genomic parts as in the wet rot fungus *Coniophora puteana* [65] or other *Saccharomyces* species [66,67], and sometimes uncovering the sterility associated with interspecific hybridization [68]. Finally, in the most extremes cases, hybridization may lead to hybrid speciation (also called homoploid speciation) as in many plant and animal taxa [69,70]. In fungi, some cases of homoploid hybrid speciation were described [71] as in the anther smut fungus *Microbotryum violaceum* (formerly *Ustilago violacea*) [72]. Indeed, the occurrence of hybridization in a given taxon gives another illustration of its tolerance to genome merging. It is not surprising that hybridization and reticulate evolution in fungi seem to occur in lineages also displaying polyploid members (figure 3) as in plants and animals [73,74].

(c) *Factors affecting genome content*

In addition to the species identified as actual polyploids (meaning that genome duplication persists in the germ line and is heritable through sexual reproduction), many other fungal species display large variation in their genome size (electronic supplementary material, table S3). In this regard, the fungal genome size database [75] provides freely accessible genome size data for more than 1000 fungal species (www.zbi.ee/fungal-genomesize). Variations in genome content may be associated with life cycle or cellular differentiation [76], such phenomena as somatic polyploidy (or endopolyploidy; box 1) rather than actual polyploidy. For example, the life cycle of *C. albicans* (the causal agent of candidiasis) is particularly atypical: *C. albicans* is a diploid yeast that has long been viewed as strictly asexual. However, a cryptic mating cycle (also referred as parasexual cycle; figure 4) has been described, through the fusion of diploid cells [77]. The resulting tetraploids then undergo random loss of multiple chromosomes, a process termed concerted chromosome loss [78]. As a consequence of such unconventional life cycle, haploid, diploid, triploid, tetraploid and aneuploid *C. albicans* populations coexist among clinical isolates [79]. *Candida albicans* may not be considered as a 'true' polyploid species (i.e. from an evolutionary viewpoint), but remains a

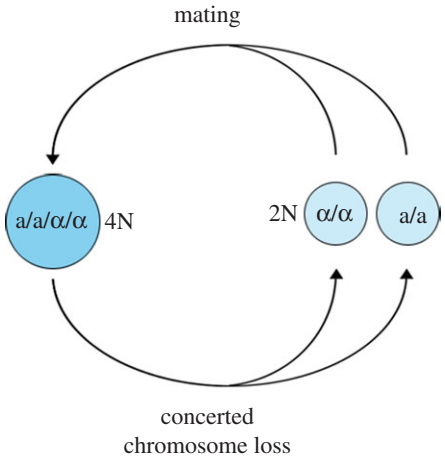

Figure 4. The parasexual cycle of *Candida albicans*.

remarkable example of variation of ploidy level associated with life cycle. A close relative of *S. cerevevisiae*, *Candida glabrata*, also displays a striking genome plasticity; although these yeasts have acquired an haploid lifestyle in comparison with other yeasts, frequent changes in the chromosomal complement have been evidenced, in relation with pathogenicity and adaptation to a fluctuating environment [80]. Thus, the yeasts exhibit a genome flexibility that may favour ploidy variations.

Several environmental factors have been shown to induce variation of genome content and chromosomal complement in various fungal species, such as heat shock [81], saline stress [82], fungicides treatments [83], host–pathogen interactions [84], etc. (electronic supplementary material, table S3). Genetic factors may also be associated with variation of genome content: some genes are associated with ploidy variation when mutated, most of these being involved in spindle body structure or function [85,86] (electronic supplementary material, table S3). Although such variations in chromosomal complements and genome size may not be considered actual polyploidy (from an evolutionary viewpoint), they are the hallmark of the genomic plasticity that may support further polyploidization and subsequent fungal speciation.

3. DIVERSIFICATION IN POLYPLOID FUNGI

Following WGD, one would expect the newly formed polyploid to possess the sum of the parental genomes and display mid-parent patterns of relative expression [87]. This so-called additivity hypothesis has been verified in cases such as that in synthetic allopolyploid cotton where 'structural genomic stasis' has been described. However, deviation from the additivity hypothesis was evidenced for many polyploid species, and duplicated genes can undergo immediate structural and functional divergence [87]. Indeed, specific patterns of evolution were described in plant and animal polyploids and are supposed to facilitate evolution and adaptation. In fungi also, WGD is associated with long- or short-term structural, functional and phenotypical diversification.

(a) *Genome evolution and diversification in polyploid fungi*

Any increase in chromosome number is expected to enhance meiotic and mitotic abnormalities, particularly

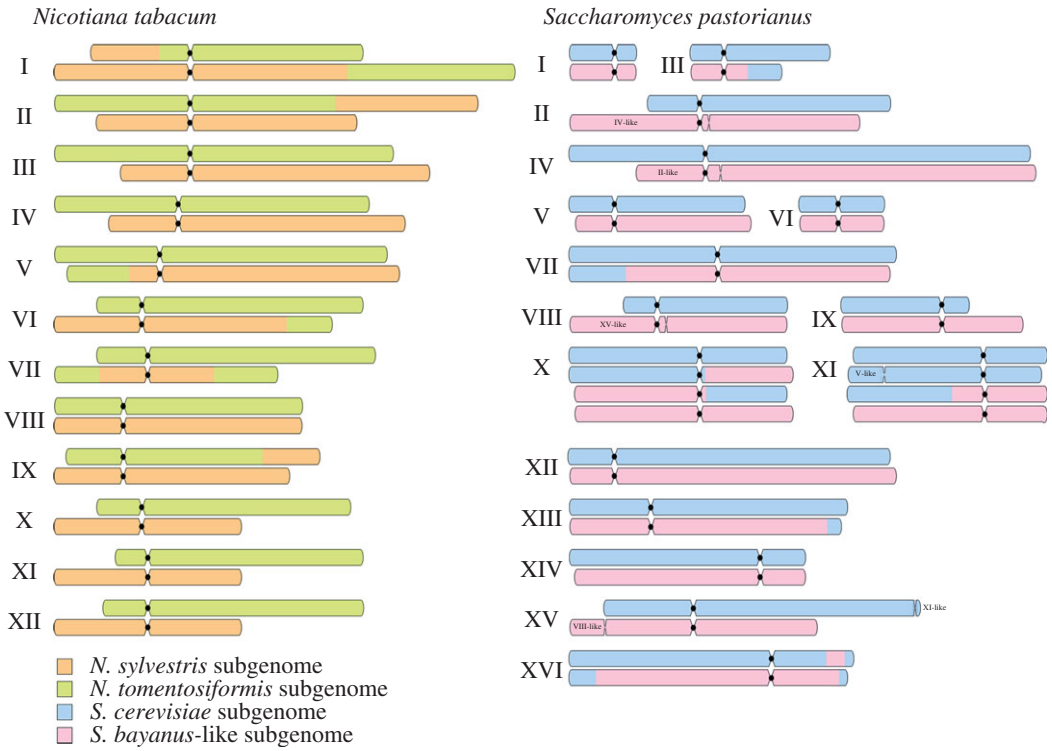

Figure 5. Polyploid genomes of *Nicotiana tabacum* and *Saccharomyces pastorianus*. The schematic genomes illustrate the parental origin of the chromosomes. The different types of chromosomes are drawn. Black circles represent centromere position. The structure of *N. tabacum* genome was adapted from Chester *et al.* [48] and displayed eight inter-genomic rearrangements. For *S. pastorianus*, Nakao *et al.* [41] identified 10 inter-genomic rearrangements and six intra-genomic translocations between chromosomes II–IV and VIII–XV (*S. bayanus*-like subgenome, now identified as *S. eubayanus*) and V–XI and XI–XV (*S. cerevisiae* subgenome).

in allopolyploid's meiosis where the chromosomal co-linearity between closely related parental genomes merged within a single nucleus may result in improper meiotic pairing and homeologous recombination [88]. Indeed, the very first meiosis of synthetic *Brassica napus* allotetraploids acts as a genome blender and generates several chromosomal rearrangements [89], and many other examples of homeologous recombination were evidenced in both plant (figure 5) and animal polyploids [90,91]. In fungi, meiotic defects were observed in yeast polyploids with general instability [92], abnormal chromosomal disjunction [93] or atypical meiotic timing and topology [94]. Genome instability in yeast polyploids was also observed during mitosis, with the occurrence of chromosome loss 30-fold higher in triploids and approximately 1000-fold higher in tetraploids than in diploids [95]. Another autopolyploid yeast series, evolving experimentally over 1800 mitotic generations, converged towards diploidy [96] mainly through chromosomal loss and some additional chromosome mis-segregation events [97]. WGD in yeast was followed by a decrease in chromosome number mainly imputable to telomere-to-telomere fusion between chromosomes [98]. Exhaustive genomic studies of the lager yeast *S. pastorianus* revealed that, following allotetraploidization, several chromosomal translocations arose between the parental subgenomes [41] as well as large chromosomal rearrangements [40,99]. These chimaeric chromosomes appear currently stable within lager strains currently used in breweries (figure 5).

Transposable elements (TEs) and other repeated sequences are traditionally involved in structural and functional dynamics of plant and animal polyploids [100,101]. They seem also involved in fungi post-polyploid evolution. TEs represent a very little part of the total *Saccharomyces* sp. genome compared with plant and animal ones: only 3 per cent of yeast genome, i.e. around 300 TE *per* haploid genome [102]. However, some translocation breakpoints in lager yeast *S. pastorianus* are located near Ty retrotransposon elements [40], suggesting that TE mediated genomic rearrangements following allotetraploidization as in other eukaryote polyploids. From a long-term perspective, the WGD event in the *Saccharomyces* lineage 100 Ma was followed by reciprocal translocations resulting from ectopic recombination between Ty elements or other repeated sequences [103]. Other well-known repeated sequences associated with genome restructuring in polyploids are the cluster of ribosomal DNA (rDNA). In plants and animals, many synthetic and natural polyploids display partial or complete homogenization of their rDNA [104–106], suggesting concerted evolution. In polyploid fungi, rDNA are also associated with genetic modifications: in the lager yeast *S. pastorianus*, one parental rDNA cluster has undergone a significant reduction following polyploidization [41] and, in *Botrytis* allotetraploid, the lack of polymorphism in the rDNA may be explained by concerted evolution [24]. The subtelomeric regions were particularly prone to duplications and rearrangements in yeasts following WGD [107] or allotetraploidization in lager yeast [108]. Genetic diversification in polyploids may involve smaller sequences and encompass limited duplication and/or gene loss as in plants and animals [109–111]. Extensive gene loss following WGD in yeast lineage is described [112] and could be a driving force of speciation. In the lager yeast *S. pastorianus*, changes in

copy number of specific repeated sequences (loss or duplication) are described, highlighting the dynamic nature of yeast polyploid genome [113]. Experimental evolution of synthetic yeast allotetraploid subjected to mutagenesis was associated with reciprocal gene loss [114]. In conclusion, polyploidization in fungi appears to be associated with various gross or restricted structural rearrangements as found in the plant and animal kingdoms. As a result, the genome of the allotetraploid *S. pastorianus* now displays highly chimaeric chromosomes that strikingly echo plant allopolyploid ones (figure 5).

(b) *Evolution of gene expression in polyploid fungi*

From a functional viewpoint, one plus one does not equal two in polyploids [87], and many plant- and animal-duplicated genomes transgress the additivity hypothesis (predicting mid-parent relative expression). In fungi, expression data in a polyploid context are available mainly within the *Saccharomyces* genus.

Microarray-based gene expression analysis of isogenic haploid, diploid, triploid and tetraploid *S. cerevisiae* strains allowed the identification of a few genes (17) displaying non-additive expression [115]. A recent analysis identified substantially more transgressive genes (65) but showed that cell size increase, rather than genome doubling itself, was the cause of gene expression alteration in yeast autotetraploids [116]. Altogether, these results suggest that genome doubling by itself may trigger few expression changes as in plant models [117,118]. By contrast, allopolyploidy in yeast is associated with several expression changes; an exhaustive expression analysis was recently conducted on *S. pastorianus* using microarray [119] and allowed to be distinguished most homeoallele pairs during the fermentation process. If 600 genes showed similar expression patterns between *S. cerevisiae* and *S. bayanus*-like (now known as *S. eubayanus*) parental genes, then 400 other homeologous genes show unequal contributions to the transcriptome of *S. pastorianus* [119]. Interestingly, the contributions of homeologous pairs vary along the fermentation process; for example, some homeoalleles display equal expression contribution at the very beginning of fermentation, and unequal contribution in the last fermentation steps [119]. Indeed, the transcriptomic profiling of *S. pastorianus* shares common features with other plant models; the unequal contributions of the homeoalleles were described, for the first time, in the allotetraploid cotton *Gossypium hirsutum* with organ-specificity [120]. Further analyses must be conducted to decipher the mechanisms underlying gene expression regulation in yeast polyploids. Functional changes may be related to the structural diversification associated with polyploidy as described in *S. pastorianus*, where a chromosomal rearrangement was coupled with a loss of function at breakpoints of the resulting hybrid *GPH1* gene [99]. Dosage compensation, a process by which genes duplicated by polyploidy or aneuploidy show diploid-like expression as described in plants [121], also counted in the functional evolution of *S. pastorianus* [113]. Epigenetic regulation of gene expression in polyploids has received a great attention in plant polyploids essentially through DNA methylation studies [122,123]. In particular, the methylation state of TE may be related to a transposition burst following polyploidization [124–126] and may be associated with the deregulation of small RNA [127]. Cytosine methylation is absent in *Saccharomyces* genus that do not possess DNA methyltransferases [128], but other epigenetic mechanisms are known; for example, histone deacetylation is involved in the regulation of gene expression in yeast [129] and it could be interesting to test its putative occurrence in polyploid and hybrid context.

(c) *Phenotypic diversification and ecological consequences*

Polyploidization triggers several structural and/or functional changes that are assumed to favour phenotypic diversification and thus facilitate further evolution and adaptation in plants and animals [1,2] and also in fungi [22,130]. In *Saccharomyces* sp., genome doubling is associated with morphological variation such as cell size, shape, organization (colonies forming) and growth [131,132]. Metabolic changes are also observed. For example, metabolic fluxes increase with the ploidy level in autopolyploid series [131,133], and the allotetraploid *S. pastorianus* and its genome donor *S. cerevisiae* display highly different exometabolomes [134]. Because the productivity of yeast cultures seems to increase with the ploidy level in many cases [135], polyploidy in *Saccharomyces* has been much more studied from a biotechnological viewpoint than from an evolutionary perspective. However, recent data regarding the fitness of polyploid yeasts were described: in soil yeasts, high ploidy level may be a mechanism of adaptation to high solar radiation [136]. Baking is closely associated with autotetraploid *S. cerevisiae*, suggesting that autotetraploidy in yeast may promote adaptation to the harsh bakery environment [33]. In *S. pastorianus* allotetraploid, specific changes in sugar and sulphite metabolism were evidenced in comparison with its *S. cerevisiae* and *S. eubayanus* progenitors [42]. These modifications may have been crucial for domestication in the lager-brewing environment [42]. The WGD in the *Saccharomyces* lineage and subsequent preferential retention of duplicated glycolytic genes may have favoured glucose fermentation, adaptation to glucose-rich environments and occupation of new ecological niches [137]. Indeed, modelling the evolution of metabolic networks in post-WGD yeasts indicates that polyploidization is generally detrimental in the original (parental) environment, but has immediate fitness benefits in new environmental conditions [138]. This may explain why autopolyploid *S. cerevisiae* strains show reduced fitness under laboratory conditions [96]. In addition, the diploidization process by itself may be adaptative: aneuploidy and major chromosomal changes in yeast may be associated with increased fitness [139]. The partitioning of yeast co-expression networks after WGD [140] led to partial redundancy and functional overlapping, and is responsible, in part, for genetic network robustness [141] that may promote adaptative changes. Further analyses of polyploid and palaeopolyploid yeasts from an evolutionary viewpoint will increase our knowledge of the consequences and the fate of duplicated genomes in fungi.

4. CONCLUSIONS AND FUTURE DIRECTIONS

Although fungal polyploidization has been long illustrated solely through yeast WGD, there is other evidence

indicating that polyploidy has played a preeminent role in the evolutionary history of the fungi kingdom, as it has in plants and animals. It is highly probable that the non-exhaustive list of past and recent polyploidization events presented here will increase greatly in the future because until now fungi are less studied than plants and animals. The cytological and phylogenetic data already available could be used to infer the evolution of chromosome number in fungi and to estimate the occurrence of polyploidy using probabilistic models as described recently [142]. Such work may help to draw a more precise image of polyploidy in fungi.

It is noteworthy that *Saccharomyces* genus emerges as the fungal alter ego of widely studied plant taxa such as the *Brassicaceae*, the *Triticeae*, etc., which exhibit a complex pattern of polyploidy. The evolutionary history of *Saccharomyces* species are shaped by past and recent WGD events, associated with hybridization and reticulate evolution. The structural and functional outcomes of polyploid *Saccharomyces* genomes strikingly reflect the evolutionary fate of plant polyploid ones, designing yeast as a relevant complementary model for polyploid studies. The yeast model may offer several technical and laboratory facilities: in addition to the high number of large-scale molecular tools available (micro-array, whole-genome sequencing, proteomics, etc.), neopolyploids can be synthesized through protoplasts fusion [143], and can be compared and/or competed with their diploid progenitor and natural auto- and allo-polyploid counterparts to analyse fitness and adaptation features. Neopolyploid yeasts are pertinent models to study reproductive isolation through the establishment of post-zygotic barriers [114] and genetic incompatibilities [144]. Yeast's short generation time (a few hours) allows experimental evolution over hundreds or thousands of mitotic generations [145] that may give new insights into polyploid evolution and subsequent diploidization. For example, experimental evolution of neopolyploids could help unravel the role of TEs in polyploid evolution and, in particular, their impact on genome modifications from both structural and functional viewpoints. Yeast polyploids could be useful to explore another interesting issue: the role of nucleocytoplasmic interactions in polyploid formation and propagation. In plants, studies of reciprocal hybrids and polyploids have evidenced differential genome evolution as in *Brassica* polyploids [146] or differences in fitness as in *Epilobium* hybrids [147]. Several authors hypothesized that the merging of two nuclear genome components with a unique cytoplasmic component in an interspecific hybrid may unbalance the interactions between the nuclei and cytoplasm, and may favour the parental genome initially associated with the cytoplasmic one, i.e. the maternal one in plants [148]. Indeed, a nuclear gene from *S. bayanus* was shown incompatible with *S. cerevisiae* mitochondria, suggesting possible nucleo-cytoplasmic incompatibilities within the corresponding hybrids [149]. Such hypothesis could be tested using yeast as a model: in most cases, hybrids resulting from crosses involving the same parents may inherit either mitochondria [63], allowing the comparison of nuclei-identical hybrids, but having different mitochondrial DNA. Moreover, it could be interesting to test the tolerance of the yeast model to high ploidy level: although natural and synthetic triploid and tetraploid yeasts have been repeatedly described, it is not known whether higher ploidy levels could be generated as in plants and animals where several dodecaploids harbouring hundreds of chromosomes are known [150–152]. Finally, there is still a lack of knowledge on the relationships between structural and functional diversification in polyploids and further adaptation ability. *Saccharomyces* yeasts are suitable biological models for systems biology approaches that will help unravel the adaptative value of WGD and understand why polyploidy is such an evolutionary success among eukaryotes.

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