Limited DNA Repair Gene Repertoire in Ascomycete Yeast Revealed by Comparative Genomics

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Abstract

Ascomycota is the largest phylogenetic group of fungi that includes species important to human health and wellbeing. DNA repair is important for fungal survival and genome evolution. Here, we describe a detailed comparative genomic analysis of DNA repair genes in Ascomycota. We determined the DNA repair gene repertoire in Taphrinomycotina, Saccharomycotina, Leotiomycetes, Sordariomycetes, Dothideomycetes, and Eurotiomycetes. The subphyla of yeasts, Saccharomycotina and Taphrinomycotina, have a smaller DNA repair gene repertoire comparing to Pezizomycotina. Some genes were absent from most, if not all, yeast species. To study the conservation of these genes in Pezizomycotina, we used the Gain Loss Mapping Engine algorithm that provides the expectations of gain or loss of genes given the tree topology. Genes that were absent from most of the species of Taphrinomycotina or Saccharomycotina showed lower conservation in Pezizomycotina. This suggests that the absence of some DNA repair in yeasts is not random; genes with a tendency to be lost in other classes are missing. We ranked the conservation of DNA repair genes in Ascomycota. We found that Rad51 and its paralogs were less conserved than other recombinational proteins, suggesting that there is a redundancy between Rad51 and its paralogs, at least in some species. Finally, based on the repertoire of UV repair genes, we found conditions that differentially kill the wine pathogen *Brettanomyces bruxellensis* and not *Saccharomyces cerevisiae*. In summary, our analysis provides testable hypotheses to the role of DNA repair proteins in the genome evolution of Ascomycota.

Key words: DNA repair, Ascomycetes, yeast.

Introduction

Ascomycetes are a diverse group of organisms with a significant impact on human beings and the environment (Fisher et al. 2012). Ascomycetes include the most severe plant pathogens (Dean et al. 2012). Aspergillosis and Candidiasis are two common human fungal diseases caused by Ascomycetes that affect millions of individuals every year worldwide (Colombo et al. 2017; Dadar et al. 2018; Maiz et al. 2018). Additionally, Ascomycete fungi are pathogenic to organisms that have significant environmental importance, such as bees and bats (Aronstein and Murray 2010; Fisher et al. 2012; Palmer et al. 2018; Simone-Finstrom et al. 2018). Ascomycete fungi also have a positive impact on humans; *Saccharomyces cerevisiae* and other yeasts are the basis of the baking, brewing, and bioethanol industries (Walker and Walker 2018).

Ascomycete fungi are continuously exposed to DNA damaging agents; the most common sources are UV irradiation and internal metabolism (Goldman et al. 2002). Pathogenic fungi are also exposed to DNA damage originated by their host, for example, the burst of Reactive Oxygen Species generated by the infected plants (Osiewacz and Stumpferl 2001; Baxter et al. 2014; Fernandez et al. 2014). DNA damage is both mutagenic and toxic because it disrupts DNA replication and transcription. Despite all of the above, very little is known about DNA repair in Ascomycetes that are not model systems.

Due to risks to genome stability and viability, all organisms developed complex mechanisms to protect cells from DNA damage (Sancar and Reardon 2004; Saini 2015; Lindahl 2016). The primary line of defense against DNA damage is DNA repair. There are often redundant mechanisms that deal with similar lesions. For example, double-strand breaks (DSBs) are highly genotoxic and mutagenic lesions; in eukaryotes, there are two mechanisms to repair DSBs—nonhomologous end joining and homologous recombination (Haber et al. 2004; Pardo et al. 2009; McKinney et al. 2013). DNA base lesions are much more common than DSBs, and they vary in their mutagenic and genotoxic potential. The majority of the

base lesions are removed by excision repair mechanisms, either base excision repair for small modifications on DNA or nucleotide excision repair (NER) for bulkier lesions (Lindahl et al. 1997). UV generates mostly two types of bulky pyrimidine dimers that are repaired by NER. The complete NER system includes DNA damage binding proteins Ddb1 and Ddb2 (Wittschieben and Wood 2003) and machinery that sends them to degradation, that is, the Cul4 pathway (Scrima et al. 2011). Two more mechanisms that repair UV lesions in addition to NER are DNA photolyase (Phr1) and UV endonuclease (UVDE). Phr1 directly reverses UV damage (Liu et al. 2011). UVDE recognizes UV lesions and makes an incision next to them. Next, flap structures are created and excised, and finally, the single strand gaps are filled in and ligated (Alleva et al. 2000; Liu et al. 2011).

There is a clear link between lesions in the DNA, malfunction of DNA repair, and chromosome aberrations, as shown in organisms from human to yeast (Lobachev et al. 2002; Wu et al. 2009; Sharma et al. 2013; Covo et al. 2014; Zeman and Cimprich 2014; Duffy et al. 2016). Decades' long observations backed-up with recent whole-genome resequencing data indicate that the karyotype of Ascomycete fungi is dynamic (Boehm et al. 1994; Dunham et al. 2002; de Jonge et al. 2013; Berman 2016). A high degree of chromosome structural and copy number variations appear among species within populations. These chromosome aberrations were found to be linked with pathogenicity in some species. In addition, they were exploited in biotechnology applications (Selmecki et al. 2006; Gresham et al. 2008; Argueso et al. 2009; Ma et al. 2010; de Jonge et al. 2013; Jones et al. 2014).

The aim of this study was to examine the diversity of the repertoire of DNA repair proteins in Ascomycete fungi. In light of the ecological diversity of Ascomycetes, particular emphasis was given to study variation in the repertoire of UV repair genes. We discovered that despite their fundamental role, the DNA repair genes' repertoire substantially varies among yeast species. Our analyses further suggest a massive loss of DNA repair genes in filamentous Ascomycetes after their divergence from the other Ascomycetes.

Materials and Methods

Data Collection

Proteome and genome files were downloaded in FASTA format from the NCBI on December 13, 2017 and were used to construct a local BLAST database. The analysis included 333 species from two Ascomycete subphyla: Saccharomycotina and Taphrinomycotina and four Ascomycete classes: Leotiomycetes, Sordariomycetes, Dothideomycetes, and Eurotiomycetes (supplementary table S1, Supplementary Material online, includes the complete list of proteome data sets).

Identifying Orthologs

Orthologous genes were determined based on a reciprocal best BLAST hit (rBBH) approach (Tatusov et al. 2000) using in-house PERL scripts. The search started with a list of 90 previously published DNA repair proteins in fungi (Goldman and Kafer 2004). All the genes in the list are experimentally validated DNA repair genes with known DNA repair activities and common gene names (e.g., UVDE, Phr1, and Rad51). First, the 90 proteins were identified in six species (*Schizosaccharomyces pombe*, *S. cerevisiae*, *Neurospora crassa*, *Aspergillus nidulans*, *Botrytis cinerea*, and *Zymoseptoria tritici*), the most studied organisms in each of the subphylum/classes. To identify orthologs of *S. cerevisiae* and *Sc. pombe*, the names of the genes were searched in the respected genome databases (SGD and Pombase [Hellerstedt et al. 2017; Lock et al. 2018)]). If the gene was not found by its name, it was identified through rBBH against *S. cerevisiae*, *Sc. pombe*, and *A. nidulans*. The *A. nidulans* orthologs were taken from the list published in Goldman and Kafer (2004) following confirmation by rBBH against the genomes of *S. cerevisiae* and *Sc. pombe*. The *N. crassa* orthologs were collected by rBBH against *S. cerevisiae* and *A. nidulans* while 30 of the genes were experimentally validated (Inoue 2011). Orthologs in *Botrytis cinerea* and *Z. tritici* were obtained by rBBH against *N. crassa* and *A. nidulans*. These six species were then used as seeds for rBBH in a total of 333 species from the subphyla of Saccharomycotina and Taphrinomycotina and the classes of Leotiomycetes, Sordariomycetes, Dothideomycetes, and Eurotiomycetes. For each subphylum/class, a species from the same group served as a seed. As cutoff for the BlastP search, the following parameters were used: *E* value of 0.1, length ratio of 0.45, and identity fraction of 0.25. Some genes were absent from the seed species. In these cases, an ortholog from another phylogenetic group was used as a seed in a BlastP search. If a hit was found in the subphylum/class, then this hit was used again as a seed in a BLAST search.

In some cases, no rBBH was found with BlastP, which may indicate either a genuine absence of the gene from the analyzed genome or an incomplete annotation. To rule out the second possibility, TBlastN searches were further performed against genomic FASTA files with the following parameters: *E* value cutoff of 10^{-5}, word size of 5, and minimum query cover of 0.8. The genome files were downloaded from NCBI on October 7, 2018. The orthologs of DNA repair genes in all species are presented in supplementary table S2, Supplementary Material online.

We manually examined no-rBBH cases. Two types of events were resolved: fusion proteins, where the ortholog in one species existed but was fused to another protein that was longer, and therefore the reverse best BLAST hit was not the original sequence. For example, the Rad51 protein of *Penicillium griseofulvum* is fused to the nuclear pore complex protein NP60. Another type of event that resulted in no rBBH

was hits through a shared domain. The best example is Rad27 and Xpg proteins that share a common domain. To resolve these conflicts, we repeated the rBBH analysis against species that were closer to the species of interest (based on the tree presented in supplementary fig. S2, Supplementary Material online).

The presence and absence data of each of the 90 proteins in the 333 species were summarized in the form of a phyletic pattern matrix, a compact representation of the data, in which each genome is a row, each column is a gene, and the *i*, *j* entry is "1" if gene *j* is present in genome *i* and "0" otherwise (Templeton 1983; Nei and Tajima 1985).

Phyletic Pattern Score Calculation

The phyletic pattern matrix was used to calculate a Phyletic Pattern Score (PPS) for each gene or protein. This score reflects the prevalence of the specific gene within a specific clade. Specifically, the PPS indicates how many species from the subphylum/class encode the specific protein. For example, the Tdp1 protein was identified in 6 out of 11 species in the Taphrinomycotina subphylum (a total of 11 species in our data set), and hence the PPS would be $6/11 = 0.55$. For each subphylum/class in Ascomycota, we also calculated the average PPS for all genes. Similarly to PPS, we calculated a score per species (number of positive hits/90) as shown in figure 1*C*.

Tree Construction and Visualization

Each of the 90 orthologous sets was aligned using MAFFT V3.705 (Katoh et al. 2009) with default parameters. Multiple sequence alignments were concatenated, and maximum likelihood (ML) trees were reconstructed by RAxML version 8.2.11 (Aberer et al. 2014; Stamatakis 2014) under the LG replacement matrix (Le and Gascuel 2008), with among-site-rate-variation accounted for by assuming a discrete gamma distribution (Yang 1994). Bipartition values of the best ML tree were estimated. Analyses were done both for the entire data set and each of the two subphyla and four classes separately. Gene presence/absence trees were visualized by a custom R script using the "ape" (version 5.3) and "hash" (version 2.2.6) R packages (https://www.r-project.org). The script was executed in R studio console version 1.1.453 (https://www.rstudio.com) using R language version 3.5.0 (https://www.r-project.org).

Mapping of Gain and Loss Events

Gain Loss Mapping Engine (GLOOME) server was used for evolutionary analysis of gain and loss events based on the phyletic pattern and the phylogenetic tree (Cohen et al. 2010; Cohen and Pupko 2011). The default parameters for the likelihood and stochastic mapping approach were applied for the estimation of the expectation for the number of gain and loss events for each DNA repair gene over the evolutionary tree. The sum of expectation values for gain and loss events of a gene is termed here the GLOOME score.

Hierarchical Clustering Analysis

The GLOOME scores were used as input to a hierarchical clustering analysis by using heatmap2 function of the "gplots" (version 3.0.1) R package. The script was executed in R studio console version 1.1.453 (https://www.rstudio.com) using R language version 3.5.0 (https://www.r-project.org). In cases where hierarchical clustering analysis was done across classes, the GLOOME scores were first ranked (highest GLOOME value was given the highest-ranked score) before applying the clustering algorithm.

Results

Creating a DNA Repair Gene Database for Ascomycete Fungi

To perform a comparative analysis of DNA repair genes, we built upon a previously published list of 90 DNA repair genes in fungi (Goldman and Kafer 2004). It is a conservative list of proteins with a known and well established DNA repair function in several organisms from *Escherichia coli* to human (Friedberg et al. 2006). Nevertheless, the list does not aim to include all DNA repair or DNA damage response genes. The genes encode either for a bone-fide enzymatic activity to repair damaged DNA or for regulators that uniquely activate DNA repair (e.g., the ATM and ATR kinases). The Ascomycete model for DNA repair in *S. cerevisiae* and several DNA repair gene lists based on this organism were published before. All the genes included in these publications were also included in our list (Eisen and Hanawalt 1999; Resnick and Cox 2000). We used the orthologs from *S. cerevisiae*, *Sc. pombe*, *B. cinrea*, *N. crassa*, *Z. tritci*, and *A. nidulans* as seeds in an rBBH search to identify orthologs of these 90 DNA repair genes in species from the subphylum of Saccharomycotina and Taphrinomycotina and the classes of Leotiomycetes, Sordariomycetes, Dothideomycetes, and Eurotiomycetes. For each subphylum/class, a species from the same class served as a seed. The orthologs used in the search are presented in supplementary table S2, Supplementary Material online.

Smaller DNA Repair Gene Repertoire in Yeasts Compared with Filamentous Fungi

Taphrinomycotina and Saccharomycotina are two yeast subphyla that diverged the earliest during the phylogeny of Ascomycetes. Later, the group of Pezizomycotina (mainly filamentous fungi) further diverged into different classes (fig. 1*A*) (Taylor and Berbee 2006; Schoch et al. 2009; Dujon 2010). Previously, it was reported that there was a burst of gene gain on the lineage leading to Pezizomycotina

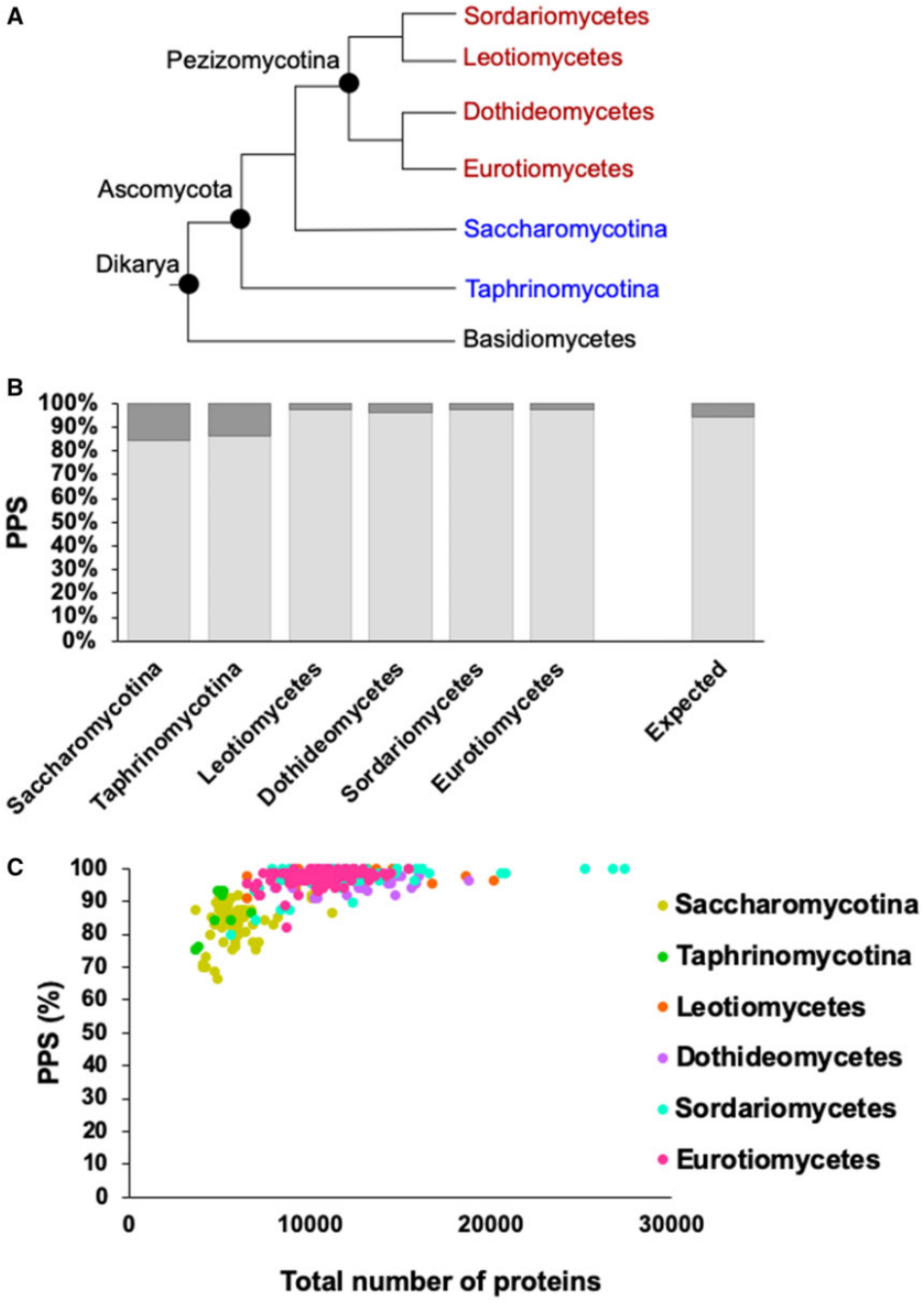

Fig. 1.—DNA repair gene PPSs in different Ascomycete classes. (*A*) Phylogeny of the Ascomycete classes studied in this work. Blue color indicates Saccharomycotina and Taphrinomycotina, which are subphyla of yeast species (mainly). Red color indicates Pezizomycotina, which is a group of classes that contains mainly filamentous fungi; in this work, we study the classes of Sordariomycetes, Leotiomycetes, Dothideomycetes, and Eurotiomycetes. In some cases, Basidiomycetes were used as a phylogenetic outgroup. (*B*) Orthologs of 90 DNA repair genes were identified in species from different Ascomycete classes using reciprocal best BlastP hit and TBlastN (see Materials and Methods). PPS per class (or average PPS) was calculated by the number of positive BLAST hits divided by the number of all possible hits (number of species × number of genes). (*C*) PPS was determined for each species and plotted against the species' proteome size; the calculation was the number of positive hits/90 (see Materials and Methods). The species are color coded by their class/subphylum.

as well as gene loss in the lineage leading to Taphrinomycotina and Saccharomycotina (Arvas et al. 2007; Nguyen et al. 2017). We studied if there were differences between the repertoires of DNA repair genes of the different subphyla/classes. Therefore, we calculated the average PPS of all genes for each subphylum/class.

We tested the hypothesis that the number of repair genes (i.e., PPS) is independent of the phylogenetic class. We found

that the association between the subphylum/class and the number of conserved repair genes is statistically significant ($X^2 = 161{,}549$, df $= 5$, $P < 0.05$). As shown in figure 1*B*, the number of conserved repair genes in Saccharomycotina and Taphrinomycotina is lower than in the other classes (fig. 1*B* and supplementary table S3, Supplementary Material online). The results shown in figure 1*B* can be explained by an inherent bias because the list of genes originated from *A. nidulans*. Therefore, we examined if there is a bias toward Saccharomycotina or Taphrinomycotina if DNA repair gene list from *S. cerevisiae* or *Sc. pombe* are analyzed, respectively. We analyzed *S. cerevisiae* DNA repair genes that were not included in our original analysis. The genes were taken from a list previously published (Aravind et al. 1999). The *Sc. pombe* genes were taken from the Pombase database. In this case, we searched the term "DNA repair" and picked genes with a bone-fide DNA repair activity. Orthologs of the yeast genes were identified in the seed species as described above, following rBBH analysis and PPS calculation. None of the genes were shown to be yeast specific. The PPS of the genes originated from *S. cerevisiae*, and *Sc. pombe* was similar between all phylogenetic groups without any bias toward Saccharomycotina or Taphrinomycotina (supplementary tables S4 and S5, Supplementary Material online). Although globally, we did not see any bias toward yeast clades, we observed several *Sc. pombe* genes that did not appear in some classes of Pezizomycotina. For example, Myh1 is absent from the classes of Leotiomycetes and Eurotiomycetes. Ast1 and Eme1 are not encoded in Dothideomycetes, and Rad60 is not encoded in Eurotiomycetes. Mag2 is a close paralog of Mag1 that is often lost during the evolution of most Ascomycete species; across all classes, few species encode for both Mag1 and Mag2 (supplementary table S5, Supplementary Material online).

It was previously reported that the number of DNA repair genes was correlated with the proteome size (Eisen and Hanawalt 1999; Acosta et al. 2015). We found a correlation between proteome sizes and PPS in Ascomycete species ($R^2 = 0.43$, $P < 10^{-14}$, Pearson least squares method) (fig. 1*C*). Most of the species with smaller proteome size and lower PPS were from Taphrinomycotina or Saccharomycotina (fig. 1*C*). We found a much lower correlation between DNA repair gene PPS and proteome size for each subphylum/class, even though proteome sizes within the groups varied considerably (supplementary fig. S1, Supplementary Material online). Therefore, it is suggested that the correlation observed between PPS and proteome size among all Ascomycetes was due to multiple loss events that occurred on the branches leading to the yeast subphylum or gain events that occurred along the branch leading to Pezizomycotina. Was the low PPS of DNA repair genes in Taphrinomycotina and Saccharomycotina due to the absence of a few genes in many species or due to the absence of many genes in only a few species? In order to distinguish between these two alternatives, we performed a hierarchical clustering analysis of all repair genes among all species examined (fig. 2). The analysis revealed that a few genes that encoded for redundant DNA repair activities were absent from many species of Saccharomycotina and Taphrinomycotina (fig. 2). We, therefore, studied the evolutionary dynamics of the genes that showed PPS lower than 50% in the subphyla of Saccharomycotina and Taphrinomycotina.

We used the gain and loss of mapping engine GLOOME with stochastic mapping (Cohen et al. 2010; Cohen and Pupko 2011). GLOOME infers the gain or loss events for each gene based on the underlying phylogenetic tree and the phyletic pattern of the gene. In addition, it provides the probability that the analyzed gene was present in each given internal node of the tree, that is, the ancestral state. Figure 3 shows examples of four genes with low subphylum PPS in Saccharomycotina and Taphrinomycotina, with their GLOOME ancestral state and prediction of gain/loss events on the different branches. In order to try and reconstruct the Ascomycete ancestral status of these four genes, we searched for orthologs in outgroup species from Basidiomycetes, Glomeromycota, Mucomycotina, and Chytridiomycota (fig. 3). We observed various alternative scenarios explaining the phyletic patterns for genes within the yeast subphyla of Taphrinomycotina and Saccharomycotina.

Some genes were specifically lost early in the divergence of Saccharomycotina. These genes are represented in figure 3 by *cul4*. *cul4* existed in high probability in the tree root and was subsequently lost in Saccharomycotina. The analysis predicted that *cul4* was maintained on the common ancestor of Saccharomycotina (N22) but was lost early in Saccharomycotina phylogeny (N27). Other genes that showed a similar pattern were *ddb1*, *polκ*, *rad4*, *mgt1*, and *uvde* (fig. 3 and supplementary table S6 and fig. S2, Supplementary Material online).

The evolutionary trajectories of the genes that are missing from Taphrinomycotina are more complex. Three examples of genes that are absent in Taphrinomycotina are shown in figure 3: *polι*, *yen1*, and *mlh2*. Each of these genes is inferred to have experienced a distinct evolutionary scenario. *polι* is missing from both Taphrinomycotina and Saccharomycotina and appears in most species of Pezizomycotina. This gene was inferred to be present in the tree root, because it was found in some species of Basidiomycota (e.g., *Ustilago maydis*, KIS71394.1) as well as in Mucoromycotina (*Bifiguratus adelaidae*) and Chytridiomycota (*Powellomyces hirtus*). However, the ancestral state in all inner Ascomycota nodes indicated that the gene was missing in all nodes but the Pezizomycotina. The status of Ascomycota ancestor (N5) is uncertain with an expectation of 0.5 for either existence or absence of the gene (fig. 3 and supplementary table S6, Supplementary Material online).

mlh2 showed a very irregular pattern in Saccharomycotina and appeared in Pezizomycotina. We were not been able to

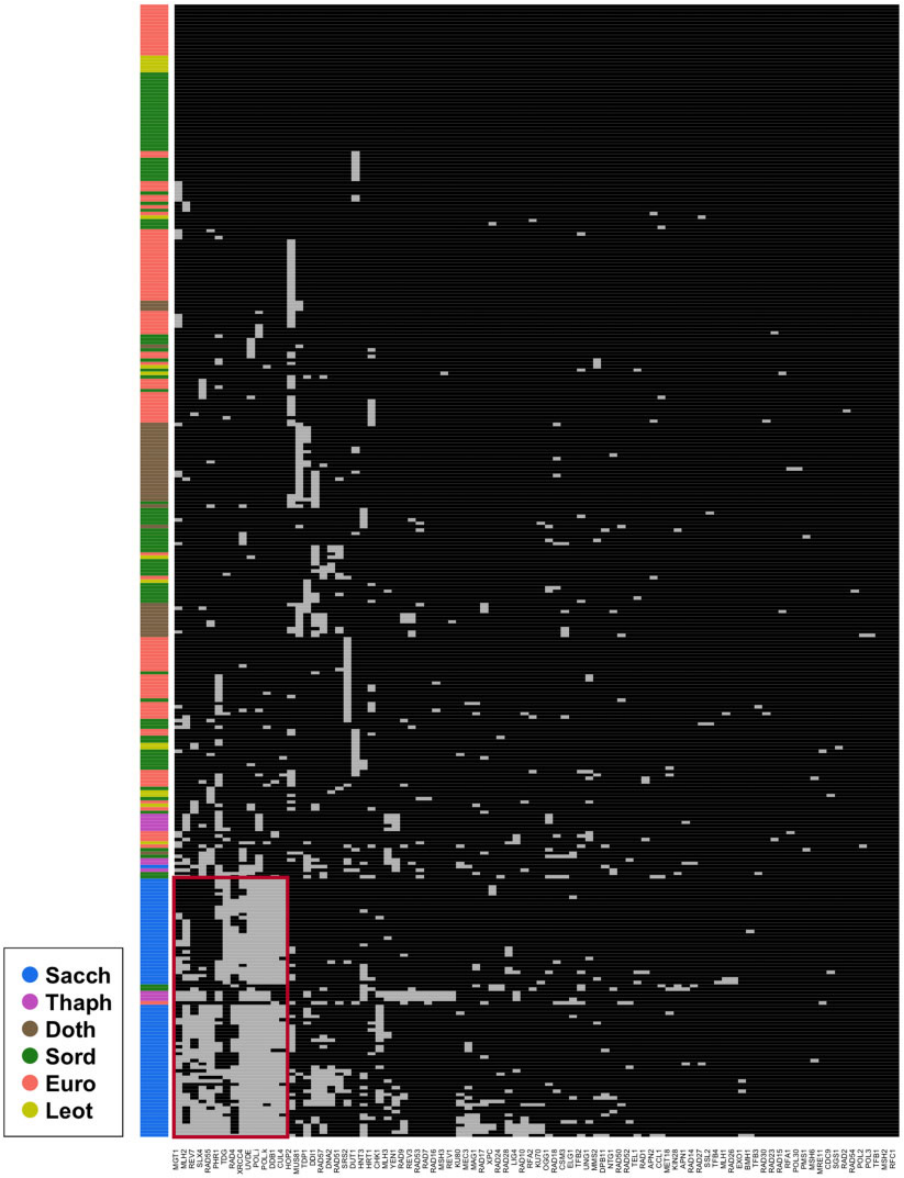

Fig. 2.—A phyletic pattern of DNA repair genes in Ascomycetes. A hierarchical clustering algorithm was used to group DNA repair genes (*x* axis) that are missing from Ascomycete species (*y* axis). Black squares represent a positive hit, and light-gray ones represent no-hit. The different classes to which these species belong to are color coded. Sacch, Saccharomycetes; Taph, Taphrinomycotina; Doth, Dothideomycetes; Sord, Sordariomycetes; Euro, Eurotiomycetes; Leot, Leotiomycetes. The genes within the red frame showed low PPS in Saccharomycotina or Taphrinomycotina and were grouped by the clustering algorithm: *mgt1*, *mlh2*, *rev7*, *slx4*, *rad55*, *phr1*, *tdg*, *rad4*, *xrcc4*, *uvde*, *polι*, *polκ*, *ddb1*, and *cul4*.

find *mlh2* in species outside Ascomycota. In contrast to *polι*, *mlh2* is missing in the tree root and is gained after the divergence of Taphrinomycotina from the common ancestor of Saccharomycotina and Pezizomycotina (N6). Saccharomycotina clade showed multiple losses (and at least one gain) of the gene (gains and losses were marked in fig. 3 only if the probability was higher than 0.7; see also supplementary table S6, Supplementary Material online).

yen1 is another gene that is missing in Taphrinomycotina. *yen1* was found in some of the Basidiomycota species (e.g., *Phlebia centrifuga*), but not in Glomeromycota, Mucoromycotina, and Chytridiomycota. *yen1* appears in many of the species of Saccharomycotina (supplementary table S6, Supplementary Material online, fig. 3, and supplementary fig. S2, Supplementary Material online). We observed the gain of *yen1* with high confidence only in Pezizomycotina (N7). Multiple gain events in Saccharomycotina did not get a high enough score. Other genes with dynamics similar to *yen1* include *xrcc4*, *slx4*, and *phr1* (supplementary table S6 and fig. S2, Supplementary Material online).

The species *Lipomyces starkeyi* and *Tortispora caseinolytica* are basal species in Saccharomycotina (Oguri et al. 2012).

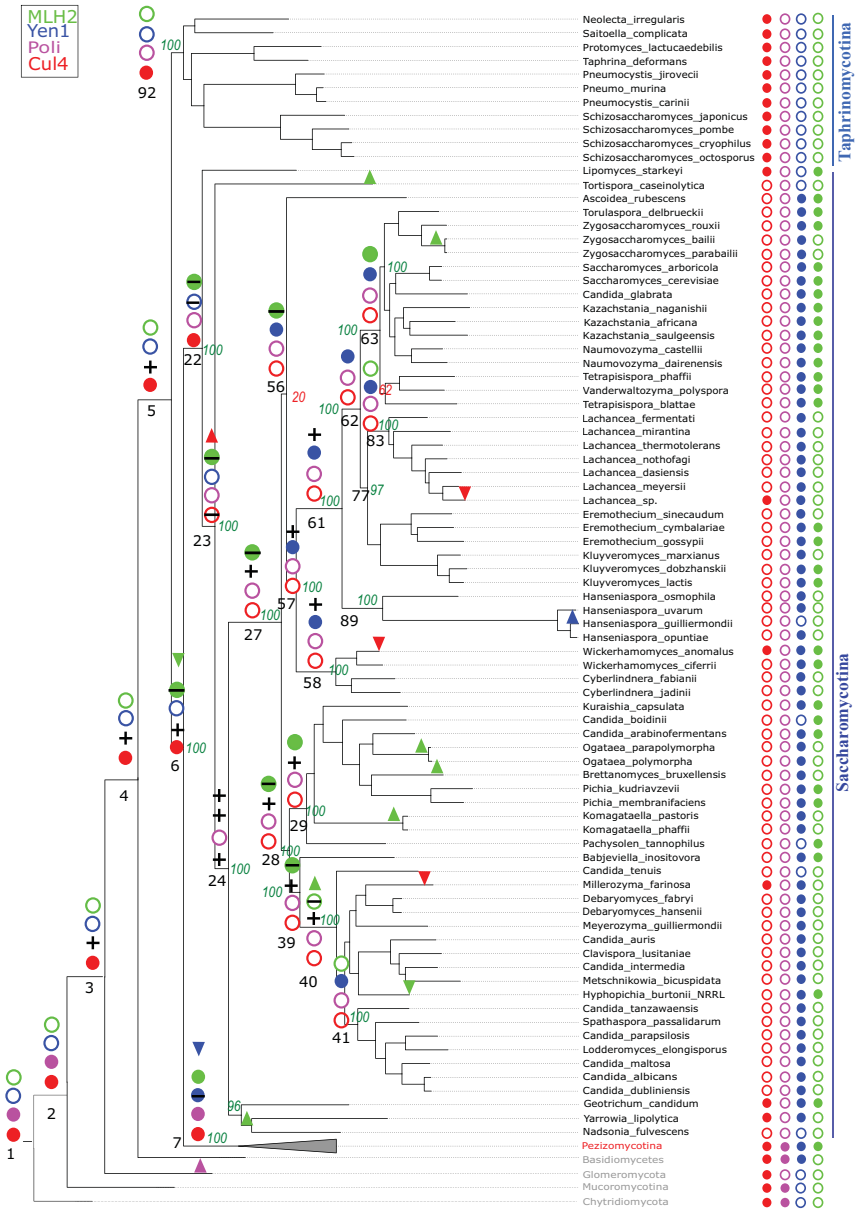

Fig. 3.—Examples of loss and gain of DNA repair proteins in Ascomycete phylogeny. Example of four proteins that show low PPS in the subphylum of Taphrinomycotina or Saccharomycotina, but high PPS in the Pezizomycotina. The low PPS is due to events that occurred along branches leading to basal nodes during the phylogeny of the subphylum. A phylogenetic tree of fungi is shown with a detailed description of Taphrinomycotina and Saccharomycotina; a schematic representation of Pezizomycotina, Basidiomycota, Glomeromycota, Mucoromycotina, and Chytridiomycota. The species that represented Basidiomycota were *Ustilago maydis*, NCBI: txid237631 and *Phlebia centrifuga*, NCBI: txid98765. The species that represented Glomeromycota, Mucoromycotina, and Chytridiomycota were *Glomus cerebriforme*, NCBI: txid658196, *Bifiguratus adelaidae*, NCBI: txid1938954, and *Powellomyces hirtus*, NCBI: txid109895, respectively. Ascomycota tree branches are according to the RaxML input tree. A filled circle represents the existence of the gene with an expectation of 0.95 or more significant, and an empty circle represents the absence of the gene with an expectation of 0.05 or lower according to GLOOME ancestral reconstruction. Expectations of 0.8–0.95 and 0.05–0.2 are shown with a line over the ancestral state; expectation values between 0.2 and 0.8 are represented with a cross. Insertion and deletions are indicated with a triangle pointing down or up reciprocally and are only shown when the probability of the event is higher than 0.7. The bipartition of the ML tree is shown for the main tree splits (in green for high support and red for low support). In red: Cul4, an example of one loss event that occurred early in Saccharomycotina. The exact branch where the event occurs is uncertain, but most probably on the branch leading to N23. In pink: Pol iota, the exact state of the ancestor of Ascomycota is unclear; in blue: Yen1, an example of a gain event on the branch leading to Pezizomycotina, as well as several gain events in Saccharomycotina; in green: Mlh2, an example of a gain that occurred at the branch leading to the common ancestor of Saccharomycotina and Pezizomycotina (N6).

Interestingly, *yen1* is missing in these two basal species, similar to the status in Taphrinomycotina. Additional genes showed the status of Taphrinomycotina rather than the rest of Saccharomycotina in *Lipomyces starkeyi* and *Tortispora caseinolytica*—for example, *cul4*, *ddb*, *polκ*, and *rad4*. Thus, the divergence of these basal species was a key point in the evolution of Saccharomycotina from DNA repair gene repertoire perspective.

Genes That Are Absent in Taphrinomycotina or Saccharomycotina Evolution Are Less Conserved in Pezizomycotina

Some genes are missing from most Saccharomycotina or Taphrinomycotina species but are present in the vast majority of Pezizomycotina species. We next tested the hypothesis that these genes are characterized by multiple gene gain and loss events within Pezizomycotina. To this end, we quantified the rate of gene gain and loss events using the GLOOME score (see Materials and Methods).

We compared the GLOOME scores of the Pezizomycotina genes that had low PPS (<50%) at the subphyla of Saccharomycotina and Taphrinomycotina to genes that had high PPS (>=50%) in these groups (fig. 2 and supplementary table S2, Supplementary Material online). The results of a one-tailed Mann–Whitney test indicated that the GLOOME score was significantly higher (and therefore less conserved) among genes that had low PPS in yeasts (fig. 4*A*, $P = 0.02$). Therefore, our hypothesis that genes that were lost early in the lineages of Saccharomycotina and Taphrinomycotina were less conserved in later divergent classes was supported.

Most of the genes with low PPS have functional redundancy with other genes that showed high PPS (whether they are paralogs or not). Figure 4*B* presents the GLOOME score versus PPS of different proteins colored by their function. Polι, Polκ, Rad30, and Rev1 are paralogs that belong to the Y family DNA polymerases that specialize in replication through damaged bases. Polι had a negligible PPS both in Saccharomycotina and Taphrinomycotina, in agreement with its very high GLOOME score. Polκ had higher PPS and lower GLOOME score. Rev1 and Rad30 that had a very high gene PPS in both groups of yeasts showed the lowest GLOOME score of all paralogs above. The same trend can be seen for mismatch repair proteins Mlh2 and its paralogs Mlh1 and Mlh3. Mgt1 and Mag1, repair methylated bases, show the same trend (fig. 4*B*). However, not always was there a correlation between the gene PPS and the GLOOME score; for example, *slx4*, *yen1*, and *mus81* function in endolytic resolution of recombination intermediates. Although *slx4* had the lowest gene PPS among them all and correspondingly highest GLOOME score, the gene PPS of *yen1* in Taphrinomycotina was very low, but it had the lowest GLOOME score in Pezizomycotina. Similar phenomena were observed among NER proteins. Both *ddb1* and *cul4* had reduced gene PPS in comparison with other NER genes, but while *ddb1* had a high GLOOME score, *cul4* had a low one (fig. 4*B*). The reason for this difference is discussed in detail below.

The DNA Repair Genes with the Lowest Conservation Encode for Redundant Repair Activities

We aimed to identify differences in the dynamics of different genes in different subphylum/class. We, therefore, determined the conservation of all proteins in each of the six groups independently. Because the number of species and the evolutionary diversity of the subphyla/classes are not uniform, we ranked the genes according to their GLOOME score within each group. Then, we applied a hierarchical clustering algorithm to the rank values of the proteins and the groups (fig. 5). We did not see classification by a repair process. Naturally, genes that are known to be essential due to their function in transcription and DNA replication are conserved in all classes (fig. 5). There is a cluster of genes with low conservation among all groups (fig. 5—marked by a black square). Interestingly, all *rad51* paralogs (*rad51*, *rad55*, and *rad57*) grouped together within this cluster. Moreover, all *rad51* paralogs grouped apart from *mre11*, *rad50*, *rad54*, and *srs2*, which are also involved in homologous recombination. *rad51* paralogs show lower conservation than *mre11*, *rad50*, *rad54*, and *srs2* (fig. 5). The meaning of this observation could be that *mre11*, *rad50*, *rad54*, and *srs2* have a stronger selective advantage than *rad51*, *rad55*, and *rad57* throughout Ascomycete evolution, which may indicate that the *rad51* paralogs, including *rad51* itself, are redundant, at least in some species.

There are two clusters (most bottom clusters in fig. 5) that show considerable variability in conservation between the classes. Dut1 is an interesting example of this cluster. The relative conservation of Dut1 among the different classes varied from very much conserved in the subphylum of Saccharomycotina to low conservation in Sordariomycetes. Specifically, high dynamics of Dut1 was observed within the *Colletotrichum* species complex (supplementary fig. S2, Supplementary Material online). More research is needed to determine the effect of this gene on genome stability in different classes.

Yeasts Show Limited Repertoire of UV Repair Genes with Implication to UV Tolerance

Abasic sites, DNA derivatives that contain only the phosphosugar backbone without any nucleotide, are common lesions caused by internal metabolism. Other common lesions are UV lesions, which are probably the most common lesions that originated from the environment. We examined if, for one of these lesions, the redundancy in DNA repair genes was kept in the subphyla of yeasts (Taphrinomycotina and

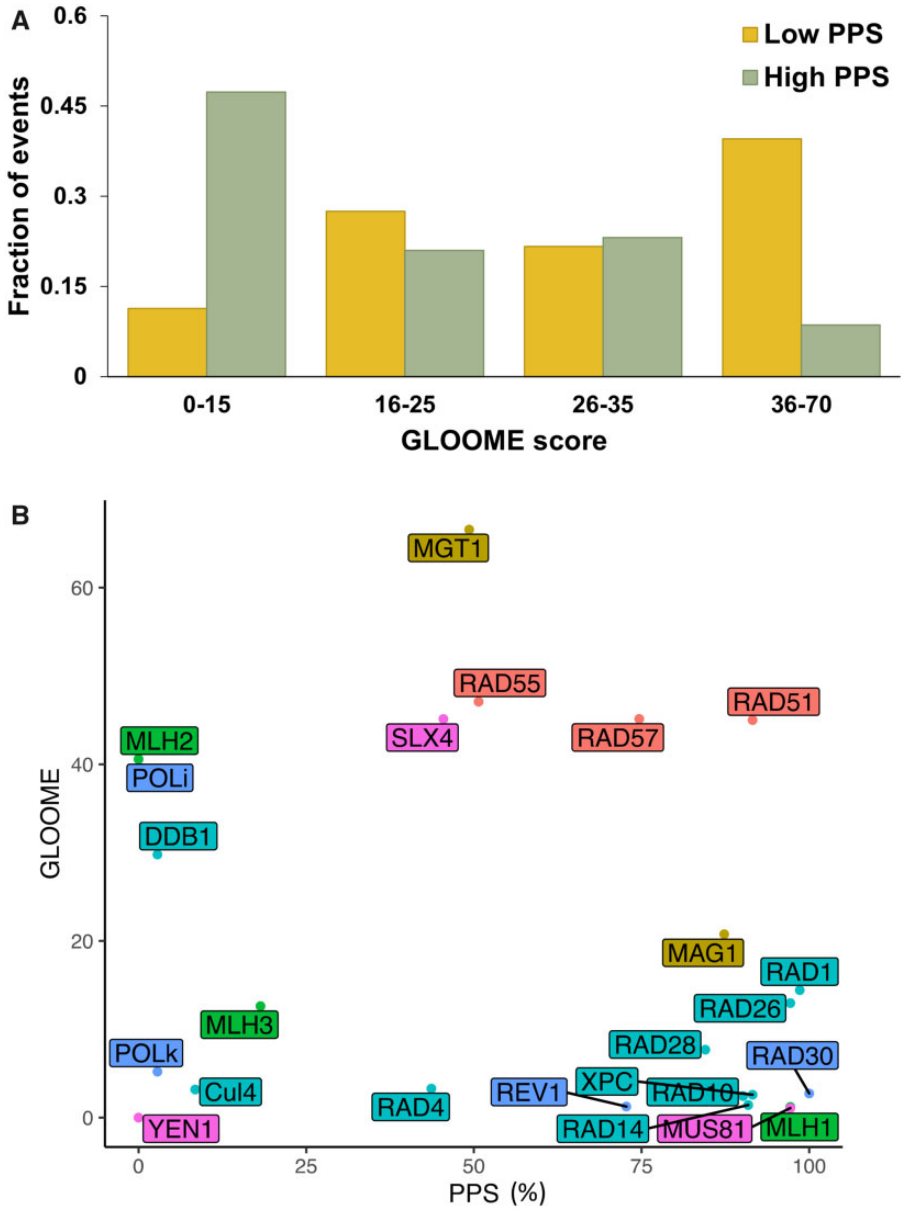

Fig. 4.—Genes with low PPS show higher evolutionary dynamics than genes with high PPS. (*A*) GLOOME score was calculated for all genes using the phyletic pattern and the tree of Pezizomycotina (fig. 1*A*). A histogram of the GLOOME score of genes with low PPS (<50%, in yellow) and high PPS (>=50%, in gray). A one-tailed Mann–Whitney test shows that genes with low PPS (<50%) in the subphylum of Taphrinomycotina and Saccharomycotina have a higher GLOOME score than genes with high PPS (>50%) in these classes (*P* value < 0.02). (*B*) A Pezizomycotina GLOOME score for selected proteins as a function of their PPS either in Saccharomycotina or Taphrinomycotina (the lowest of them) is shown. Proteins are grouped by their function (color coded).

Saccharomycotina). There are three enzymes that process abasic sites; Apn1, Apn2, and Ntg and two enzymes that process UV lesions specifically: UVDE and Phr1 (NER repairs UV lesions but is not specific to UV). We compared the PPS of genes that repair specifically UV lesions or abasic sites and found that in yeasts, the PPS for each of the enzymes that repair abasic sites was higher than the one of UV repair genes (fig. 6*A*). This indicates that at least early in the divergence of yeast the driving force to have redundant UV repair pathways was smaller than abasic sites.

Our analysis not only allows investigation into the evolution of DNA repair genes but also predicts the vulnerability of fungi to specific DNA damage based on the absence of DNA repair genes. Filamentous fungi, in general, are resistant to UV

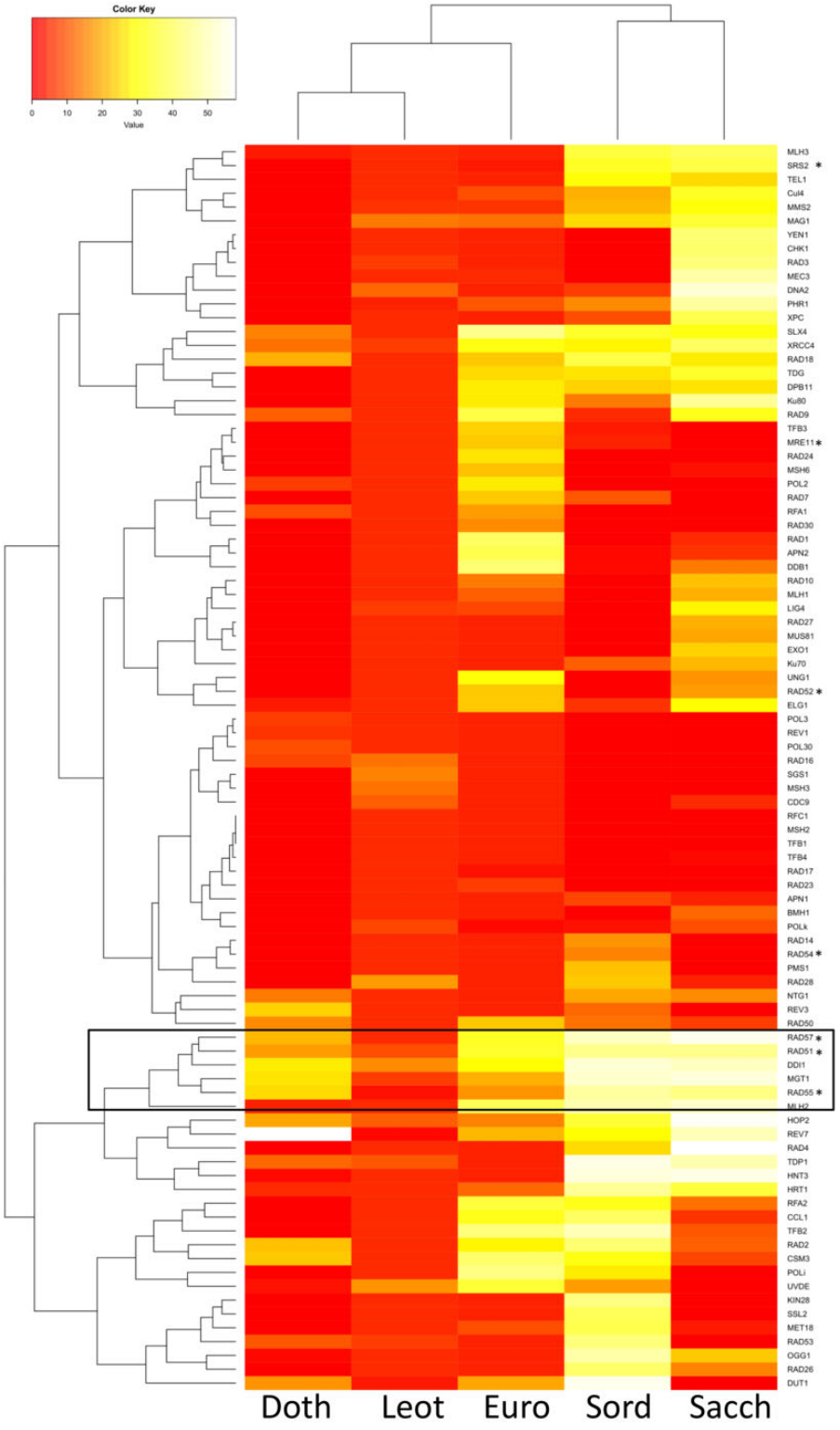

Fig. 5.—Rad51 paralogs are less conserved than other genes involved in recombination. The GLOOME score for all 90 DNA repair genes was calculated independently for the groups of Saccharomycotina, Sordariomycetes, Leotiomycetes, Dothideomycetes, and Eurotiomycetes. Next, for each class, the genes were ranked by their GLOOME score—the higher the GLOOME score, the higher the rank and the lower the conservation. A hierarchical clustering algorithm was used to group the proteins according to their rank values in the different groups. Black square encloses a group of genes that show low conservation in all classes, among them all Rad51 paralogs, including Rad51 itself. These paralogs are grouped apart from other proteins involved in recombination (marked with an *).

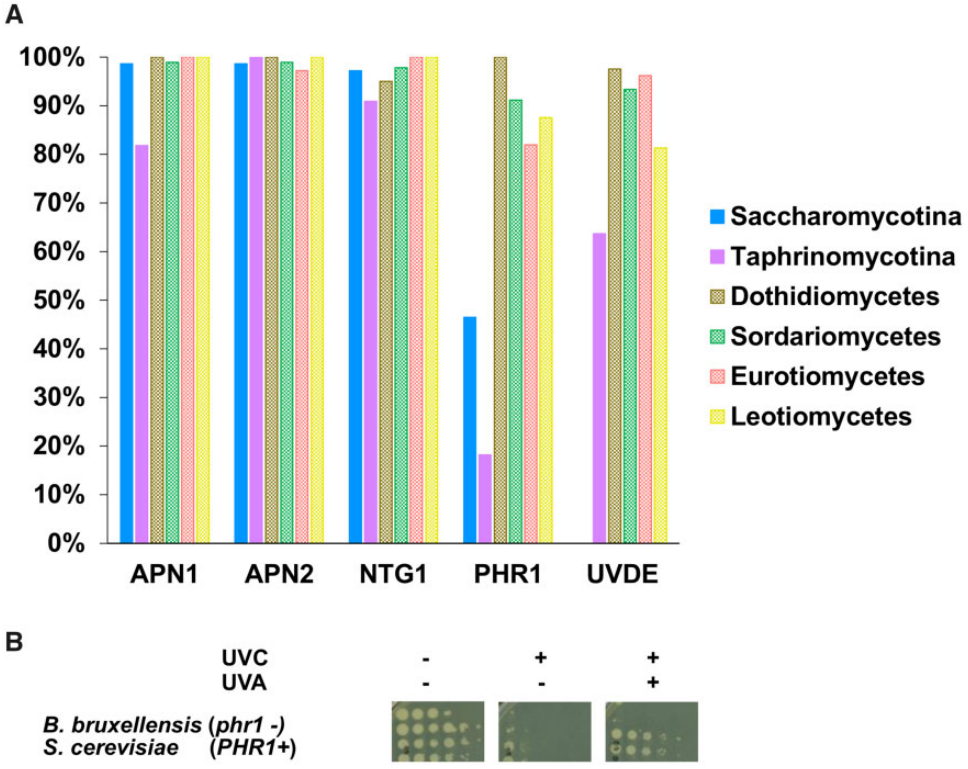

Fig. 6.—Saccharomycotina loss of UV-specific repair genes. (*A*) The PPS of each Ascomycete class for genes that encode proteins that are involved in the repair of abasic sites (Apn1, Apn2, and Ntg1) and proteins that are involved specifically in UV repair (Phr1, UVDE) in Saccharomycotina and Taphrinomycotina is shown. Classes are color coded. Solid bars represent yeast subphyla (Saccharomycotina and Taphrinomycotina), whereas striped bars represent Pezizomycotina classes (Dothideomycetes, Sordariomycetes, Eurotiomycetes, and Leotiomycetes). (*B*) *Saccharomyces cerevisiae* (*PHR1*) and *Brettanomyces bruxellensis* (*phr1* null) cultures were pronged to YPD plates and either irradiated or not with 120 J/m^2 UV-C. Next, the plates were either exposed or not for another 60 min to UV-A (activates direct reversal repair of UV lesions by the Phr1 enzyme).

because they encode for a complex NER system, UVDE, and photolyase (Phr1) (Goldman et al. 2002). As we described earlier, Saccharomycotina had lost at the common ancestor the UV repair gene UVDE and the Cul4 and Ddb1 components of NER. Therefore, they are left with a basic NER machinery and photolyase (supplementary fig. S2 and table S2, Supplementary Material online). Some of the Saccharomycotina species also lost photolyase (Phr1). In order for Phr1 to be active, a UV-A light is needed, in a process known as photoreactivation. The fungi that lost Phr1 are predicted to be UV sensitive, especially under conditions of photoreactivation.

Brettanomyces bruxellensis is a food and wine spoilage yeast that lost *phr1*. In several food-tech niches, *B. bruxellensis* competes with *S. cerevisiae*, which encodes for an active *phr1* gene. We examined if we can selectively kill *B. bruxellensis* by exposing yeast to UV allowing photoreactivation. To this end, *S. cerevisiae* and *B. bruxellensis* were irradiated with UV-C that caused lesions in the DNA. Next, we illuminated both species with UV-A that hardly damages DNA but activates Phr1 (photoreactivation). The ability of the two species to form colonies following the described treatment was measured. *Saccharomyces cerevisiae* was shown to be much more resistant to UV lesions than *B. bruxellensis*, especially under photoreactivation conditions (fig. 6*B*).

Discussion

We determined here the DNA repair repertoire of the major players in Ascomycete fungi (supplementary table S2, Supplementary Material online). We note that sequencing gaps and errors, along with misannotations and the rapid evolution of some genes, can lead to false negative results. We manually inspected ambiguities of our orthologous identification pipeline, thus providing an accurate database of DNA repair proteins in Ascomycetes.

We found that several genes were lost along the branches leading to the subphyla of Saccharomycotina and Taphrinomycotina. Other genes were gained along the branch leading to Pezizomycotina (figs. 1–3—genes with low PPS, supplementary tables S3 and S6, Supplementary Material online). Our findings are in agreement with previous publications demonstrating loss of genes on branches leading to Saccharomycotina or Taphrinomycotina or gain in the

common ancestor of Pezizomycotina (Arvas et al. 2007; Nguyen et al. 2017). A very clear example of gene loss is the loss of DNA polymerase Kappa from Saccharomycotina (supplementary table S6 and fig. S2, Supplementary Material online). Orthologs of this gene are found in bacteria as well as in fungi, flies, and mammals (Jarosz et al. 2006). Another interesting example is the loss of Mus81 from Dothideomycetes (supplementary fig. S2, Supplementary Material online), a conserved resolvase (West and Chan 2017).

The genes with low PPS encoded mostly for enzymatic activities that are redundant in the cell. Importantly, our analysis focused on genes that are known for either their DNA repair activity or their unique capacity to activate DNA repair. Other genes, which do not encode for enzymes that repair damaged bases in the DNA or are not crucial to activate such enzymes but may facilitate the ability of the cell to protect against DNA damage, were not included. Other types of genes that were not included are genes that contribute to DNA repair but have another independent activity in the cell. For example, Fumarase is a key enzyme in the tricarboxylic-acid-cycle but is also involved in DNA repair (Yogev et al. 2010). As recently published, the list of genes that may support DNA repair is not final (Sherill-Rofe et al. 2019). It is possible that there are yeast-specific DNA repair facilitator genes that improve the overall capacity of DNA repair in these organisms despite reduced gene repertoire of DNA repair genes per se. As a group, the genes with low PPS in Saccharomycotina and Taphrinomycotina tended to be lost during the phylogeny of Pezizomycotina more than their high PPS counterparts (fig. 4). One interesting example is the Y family translesion DNA polymerases (*rad30*, *rev1*, *polκ*, and *polι*) specialized in bypassing damaged bases. *polι* is absent in Taphrinomycotina and Saccharomycotina, while *polκ* was lost from Saccharomycotina. These four paralogs are conserved through mammals. Our analysis indicated a hierarchy in conservation, where the high PPS paralogs *rev1* and *rad30* were shown to be more conserved in Pezizomycotina (fig. 4*B* and supplementary fig. S2, Supplementary Material online). Interestingly, this conservation pattern is in good agreement with functional data originated in mammals that show a stronger phenotype for mice mutated in *rad30* and *rev1* comparing with *polι* mutants (for a summary see Yamanaka et al. [2017]).

In general, we found that genes that had a low PPS in Saccharomycotina and Taphrinomycotina showed high gain and loss dynamics (high GLOOME score) in Pezizomycotina. However, *cul4* and *yen1* showed low PPS in Saccharomycotina or Taphrinomycotina and low GLOOME score (high conservation) in Pezizomycotina (fig. 4). Cul4 functions in NER through facilitating the degradation of Ddb1 after the DNA damage is recognized, and the NER machinery is recruited to the lesion site (Moser et al. 2005; Scrima et al. 2011). In Pezizomycotina, the conservation of Cul4 is higher than several other NER proteins (fig. 4); this could be explained by the broad spectrum of functions Cul4 participates in. Cul4 is an adaptor of E3 ubiquitin ligase, which in fungi is involved in several different chromosome and chromatin transactions such as DNA methylation and histone monoubiquitination (Moss et al. 2010; Zhao et al. 2010; Kuscu et al. 2014; Adhvaryu et al. 2015; Zeng et al. 2016). Although both Cul4 and its partner Ddb1 are lost at the branch leading to Saccharomycotina, Cul4 is much more conserved than Ddb1 in Pezizomycotina. The possible explanation is found in the nature of the Cul4-Ddb1 complex, a scaffold that brings together proteins to be tagged with ubiquitin by ubiquitin ligase. There are multiple possible partners in this complex scaffold where Cul4 might be the central player (Lee and Zhou 2007). If the Cul4 pathway is highly important for chromosome biology, how come it is lost from Saccharomycotina? Rtt101 and Mms1 are *S. cerevisiae* proteins that function in some, but not all, aspects similarly to Cul4-Ddb1, despite the fact they show very low sequence similarity to Cul4 (Han et al. 2013). To the best of our knowledge, the Rtt101-Mms1 complex does not function in damage recognition as part of NER as Cul4-Ddb1. Hence, Saccharomycetes probably lost Cul4-Ddb1 regulation of NER but still had Cul4-like functions that were important for other pathways. Yen1 was lost entirely from Taphrinomycotina but was one of the most conserved proteins in Pezizomycotina. At this point, we cannot explain this observation.

What is the connection between the DNA repair repertoire and organism capacity to repair lesions in the DNA? Several examples show a direct correlation between the repertoire of UV repair genes and a species' ability to sustain UV damage. Saccharomycotina lost two modules of UV repair: the UVDE, also known as an alternative to NER, and Ddb1-Cul4 proteins that control the canonical NER pathway. Some Saccharomycotina species, such as *B. bruxellensis*, also lost Phr1. We were able to show that *B. bruxellensis* was significantly more sensitive than *S. cerevisiae* under the condition of photoreactivation (fig. 6*B*). Similarly, it was previously shown that *Pseudogymnoascus destructans* is more sensitive to UV than its sister taxa *Pseudogymnoascus verrucosus* due to lack of both UVDE and Phr1 (Palmer et al. 2018). *Pseudogymnoascus destructans* is also more sensitive to methyl methanesulfonate than its sister taxa, probably due to loss of UVDE and Mag1 (supplementary table S2, Supplementary Material online).

The repertoire of DNA repair genes may not always predict the capacity of repair. Our analysis indicated that the resolvase gene *mus81* was lost at the common ancestor of Dothideomycetes (supplementary fig. S2, Supplementary Material online). Mus81 partner, Eme1, is also absent in Dothideomycetes (supplementary table S5, Supplementary Material online). Mus81 is a conserved and important protein in Ascomycetes and Eukarya; it functions in the resolution of

recombination intermediates (Interthal and Heyer 2000; Inoue 2011; Wyatt and West 2014). Although Mus81 is important for recombination, species from Dothideomycetes are proficient in meiotic and mitotic recombination, as observed by efficient gene replacement and population diversity (Zwiers and De Waard 2001; Ganem et al. 2004; Eliahu et al. 2007; Stukenbrock and Dutheil 2018). *Saccharomyces cerevisiae* and *N. crassa* mutants that lost Mus81 are very sensitive to DNA damage, and therefore, the ability to withstand DNA damage in Dothideomycetes should be further examined. More specifically, the mechanism of recombination in the absence of Mus81 should be further investigated in *Z. tritici* and *Cochliobolus hetrobulus*, two Dothideomycetes that are known to be recombination proficient (Ganem et al. 2004; Eliahu et al. 2007; Croll et al. 2015; Stukenbrock and Dutheil 2018).

Does the loss of DNA repair genes in Ascomycetes have an ecological or evolutionary footprint? We could not find a clear connection between the UV repair gene repertoire and the determination of the ecological niche. *Saccharomyces cerevisiae* has a similar niche to *N. crassa*, though the latter encodes for UVDE and has a better UV-C survival (Goldman et al. 2002). *Fusarium oxysporum* is a soil-borne fungus but still encodes for three functional UV repair mechanisms.

Loss of DNA repair genes, not only UV repair, might lead to an increase in the rate of point mutations that can be observed as longer branches in a sequence-based tree. However, in general, we do not see a significant difference in the length of the branches in the Saccharomycotina and Taphrinomycotina clades when compared with Pezizomycotina. Although not precisely determined, it seems that the branch leading to the genus *Hanseniaspora* is significantly longer than other branches in the tree, in agreement with the lowest PPS in Saccharomycotina (supplementary fig. S2 and table S2, Supplementary Material online). It was recently reported that *Hanseniaspora* species lost multiple DNA repair genes and are indeed mutators (Steenwyk et al. 2019).

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

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