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Minireview

MINIREVIEW

# Hybridization and the origin of new yeast lineages

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**One sentence summary:** Here, the consequences of hybridization at the genetic and ecological levels are discussed.

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## ABSTRACT

Hybrids originate from the mating of two diverged organisms, resulting in novel lineages that have chimeric genomes. Hybrids may exhibit unique phenotypic traits that are not necessarily intermediate between those present in the progenitors. These unique traits may enable them to thrive in new environments. Many hybrid lineages have been discovered among yeasts in the Saccharomycotina, of which many have industrial or clinical relevance, but this might reflect a bias toward investigating species with relevance to humans. Hybridization has also been proposed to be at the root of the whole-genome duplication in the lineage leading to *Saccharomyces cerevisiae*. Thus, hybridization seems to have played a prominent role in the evolution of Saccharomycotina yeasts, although it is still unclear how common this evolutionary process has been during the evolution of this and other fungal clades. Similarly, the evolutionary aftermath of hybridization, including implications at the genomic, transcriptional, physiological or ecological levels, remains poorly understood. In this review, I survey recent findings from genomic analysis of yeast hybrids of industrial or clinical relevance, and discuss the evolutionary implications of genomic hybridization for the origin of new lineages, including when such hybridization results in a whole-genome duplication.

**Keywords:** hybridization; whole-genome duplication; allopolyploidization; yeast; *Saccharomyces*; *Candida*

## INTRODUCTION

Sexual reproduction is a hallmark of eukaryotic organisms, and is predicted to have been already present in the LECA, the Last Eukaryotic Common Ancestor (Koumandou *et al.* 2013). This trait is associated with several advantages such as genetic recombination, which allows purging deleterious mutations or producing optimal combinations of beneficial mutations more efficiently (Crow 1994). Mating is generally a highly controlled process, with mechanisms that are in place to ensure that it only occurs at specific circumstances, and between related organisms. Additionally, there may be other barriers to sexual reproduction between non-related organisms, which prevent the formation of zygotes (pre-zygotic barriers) or render them non-viable (post-zygotic barriers). However, these barriers can sometimes be surpassed, which results in the formation of hybrids. Hybrids can show unique traits that are not necessarily intermediate between those of their parents, and which can provide a selective advantage in a given environment—a phenomenon also known as hybrid vigor. Hybrids have been traditionally studied from the perspective of macro-organisms, such as animals and plants, where morphological traits can be used to distinguish between species and, eventually, to find intermediate forms representing hybrids. In microbial organisms,

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such as yeasts, distinct species may have few or none morphological differences, and although they can exhibit physiological differences, these may as well vary widely among strains of the same species. This makes the identification of hybrids from morphological or physiological traits a very difficult task. As a result, the presence of yeast hybrids went unnoticed until the development of *Saccharomyces cerevisiae* as a research model for genetics and biochemistry. The use of metabolic traits—such as fermentation capabilities—as markers in crosses between strains allowed the discovery that several *Saccharomyces* strains, initially described as independent species, were actually hybrids (Dujon 2012; Morales and Dujon 2012; Hittinger 2013; Leducq *et al.* 2016). More recently, the advent of genome sequencing and its broad use among Saccharomycotina has revealed that hybridization is pervasive across the whole clade (see Fig. 1, and sections below). Hybrid yeasts have been commonly found in industrial environments such as those involving fermentation of different products (Hellborg and Piskur 2009; Curtin *et al.* 2012; Morales and Dujon 2012; Borneman *et al.* 2014; Walther, Hesselbart and Wendland 2014; Smukowski Heil *et al.* 2018), and more recently they have been increasingly reported among clinical isolates (Pryszcz *et al.* 2014, 2015; Schröder *et al.* 2016; Mixão and Gabaldón 2018; Mixão *et al.* 2019). Furthermore, yeast hybrids have also been identified in natural environments (Leducq *et al.* 2016). All these cases suggest that hybridization is a powerful driver for adaptation to novel environments. Indeed, the fact that most described Saccharomycotina hybrids are clinically relevant or industry-relevant species might reflect an anthropocentric bias in studying the organisms with a more direct impact. In this regard, Saccharomycotina hybrids have been found to be common in some environments such as insect guts (Stefanini *et al.* 2016; Varela *et al.* 2019), suggesting a possible role of insects in facilitating mating between different lineages and the dispersal of the resulting hybrids (Madden *et al.* 2018). A reasonable hypothesis is that yeast hybrids should be common in other environments, particularly those with extreme or unusual conditions.

The term hybridization has been used in biology to define lineages resulting from the crossing of genetically distinct organisms, without necessarily referring to the cross of different species. For instance, in his famous experiments to determine the laws of heredity, Gregor Mendel used the word hybrid to refer to the progeny resulting from crossing different breeds of the same species of peas—*Pisum sativum* (Mendel 1866). Later, however, the definition of hybrids has been commonly linked to the concept of species, differentiating between intra- (within) or inter- (across) species hybrids, and recognizing the role of hybridization in the origin of new species (Abbott *et al.* 2013). This framework, however, transposes all the problems of the species concept, which is particularly contentious for microbial organisms, to the concept of hybridization. Hence, given the inherently challenging issue of the application of the species concept in microorganisms, the definition of hybridization in yeasts is necessarily fuzzy. In the context of this review, I will consider hybrids as organisms or lineages resulting from the mating of two other lineages that are sufficiently divergent from each other so that barriers to sexual reproduction are significant, preventing continuous gene flow between the two considered lineages, and making the existence of genetic incompatibilities likely. Based on this, I propose to consider three different *hybrid genetic zones* (Fig. 2). For Saccharomycotina, we have earlier proposed the use of levels of nucleotide sequence divergence higher than 1% as an appropriate threshold to define hybridization, not necessarily inter-species, particularly if other genomic patterns point to highly restricted gene flow between these two lineages (Naranjo-Ortiz and Gabaldón 2020). However, most of the examples discussed below concern hybridization between lineages separated by larger evolutionary distances.

## INDUSTRIAL HYBRIDS

Industrial yeasts were among the microbial species in which hybrids were first recognized. Already in the late 80 s DNA reassociation studies recognized the hybrid nature of a *Saccharomyces* yeast used in the production of lager beer: *S. pastorianus* (Vaughan Martini and Martini 1987). As molecular sequencing technologies evolved and more strains were analyzed, the number of identified Saccharomyces hybrids increased, with hybrids being found in different fermentations of wine, cider, beer or other beverages (Libkind *et al.* 2011; Morales and Dujon 2012; Hittinger 2013; Peris *et al.* 2018; Gallone *et al.* 2019; Langdon *et al.* 2019). Different hybrids of the *Saccharomyces* species complex have adapted to environments other than alcoholic fermentations such as olive brine, where adaptation to the artificial environment of the yeast seem not have any direct benefit from the human perspective (Pontes *et al.* 2019). Beyond *Saccharomyces*, many hybrids of industrial interest have been identified in other Saccharomycotina (Fig. 1), including *Metschnikowia* (Piombo *et al.* 2018), *Zygosaccharomyces* (Solieri, Cassanelli and Giudici 2007), *Dekkera* (Curtin *et al.* 2012), *Pichia* (Smukowski Heil *et al.* 2018), *Millerozyma* (Louis *et al.* 2012), and *Saccharomycopsis* (Choo *et al.* 2016). Hybrids are commonly found in industrial environments, probably reflecting that hybridization is an efficient mechanisms driving adaptations to new environments. In fact hybridization has been proposed as a strategy to optimize desired traits, such as cryotolerance in wine fermenting yeasts (Gibson *et al.* 2017).

Some of the environments in which hybrids have been identified have in common that they have been selected by human, in some cases for centuries. This has led to processes of microbial domestication in which humans, by the creation of a new niche, the propagation of this niche, and the selection of particular properties have inadvertently selected strains with unique traits that are present in that niche and confer to it the desired properties. However, hybrid lineages that initially colonized these human-related environments, must have originated spontaneously from the crossing of species that were present in the surrounding environment or the raw materials used to prepare these products. Indeed hybrids have been shown to form spontaneously in natural environments in several yeast genera including *Saccharomyces* (Alsammar and Delneri 2020) and *Zygosaccharomyces* (James *et al.* 2005). Human-made industrial environments may favor the formation of new hybrids by bringing together products from different environments or from different geographical regions and therefore putting in close contact species that were previously geographically distant.

## PATHOGENIC HYBRIDS

In recent years, the hybrid nature of several human pathogenic yeasts within the Saccharomycotina has been uncovered (Mixão and Gabaldón 2018). These hybrids are generally diploid organisms that cannot reproduce sexually and whose hybrid origin is denoted by the presence of high levels heterozygosity in their genomes. Within Saccharomycotina, the first human yeast pathogens to be identified as hybrids belong to the *Candida parapsilosis* species complex. This species complex, initially defined as a single species, *Candida parapsilosis*, was subdivided in three

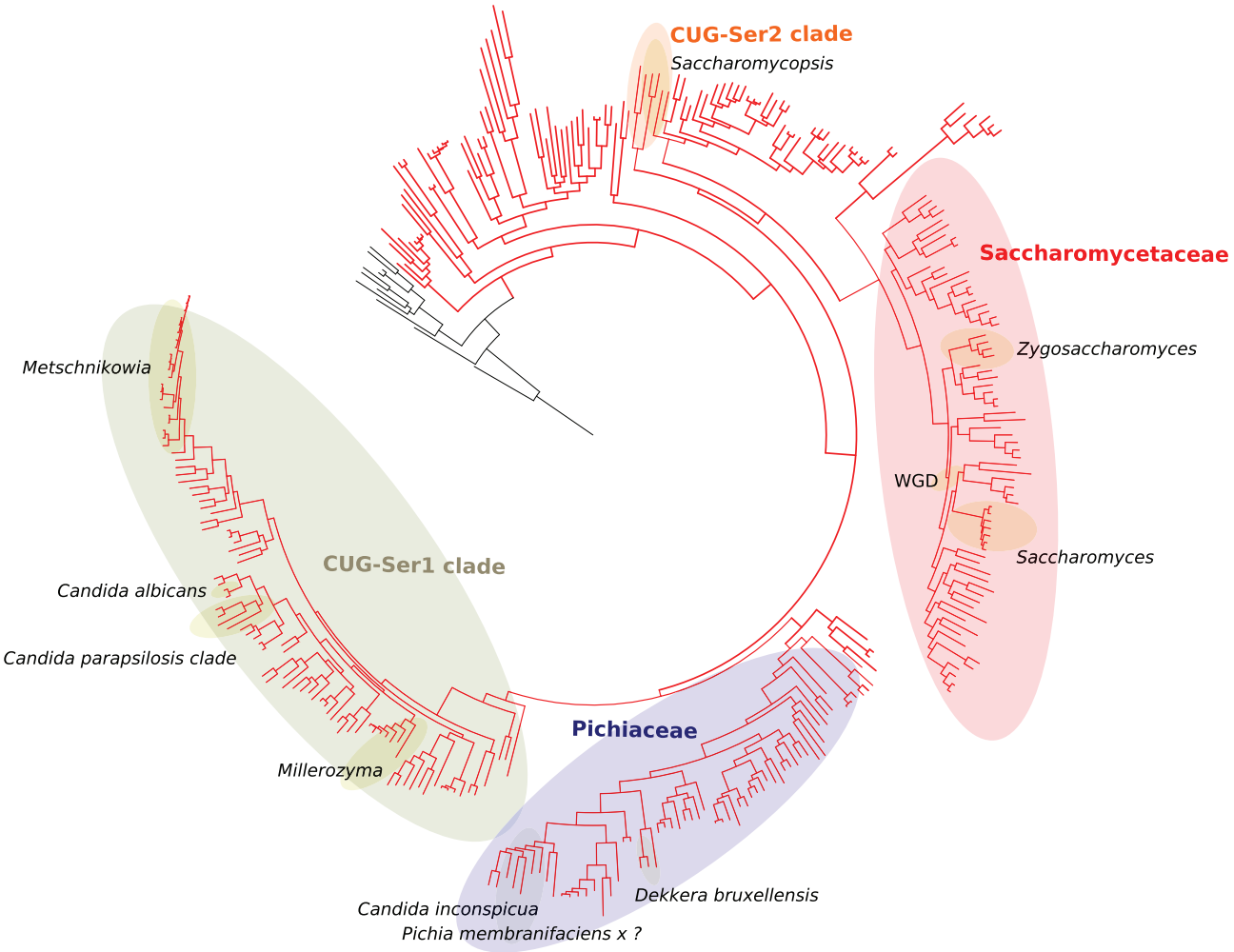


**Figure 1.** Phylogenetic diversity of Saccharomycotina hybrids. The phylogenetic diversity of Saccharomycotina is represented according to a recent phylogenetic study of sequenced genomes (Shen *et al.* 2018). Major clades where the presence of hybrids has been reported are indicated with colored ellipses. Species and lineages where hybridization has been reported are marked with smaller brown ellipses and the respective name of the species, genus or lineages. Whole-genome duplication (WGD) denotes the ancestral WGD that resulted from an allopolyploidization event (Marcet-Houben and Gabaldón 2015). *Candida albicans* is included, as it has been recently proposed to derive from an ancestral hybrid based on its genomic features (Mixão and Gabaldón 2020). Major clades are as defined in Shen *et al.* (2018). CUG-Ser1 clade includes interspersed taxa from the families Debaryomycetaceae, Metschnikowiaceae and Cephaloascaceae; the CUG-Ser2 clade includes the families Ascoideaceae and Saccharomycopsidaceae; and the Pichiaceae clade includes several taxa in need of reassignment. Studies where the depicted hybrids are discussed are cited in the main text.

distinct species in 2005: *Candida parapsilosis*, *Candida orthopsilosis* and *Candida metapsilosis*, on the basis of genetic differences (Tavanti *et al.* 2005). The hybrid nature of some *C. orthopsilosis* strains was revealed when their genome was sequenced and the genomic reads mapped to a previously sequenced homozygous strain (Pryszcz *et al.* 2014). The read mapping analysis indicated the existence of homozygous regions interspersed by regions with high heterozygosity corresponding to an allelic divergence of 5% at the nucleotide level. Importantly, the study found that two strains from geographically distant regions (Singapore and the United States) were virtually identical in terms of overlap of homozygous and heterozygous blocks, suggesting a recent common origin. Altogether, these results indicated that a hybrid between two lineages that were 5% divergent was formed, and that this hybrid successfully spread, being the cause of infections at very distant locations. Based on these findings, we hypothesized that the hybrid may have had some enhanced capacity of dispersion or a higher virulence potential than non-hybrid strains.

At that time, though, this idea was little more than wild speculation. The sequencing of 11 *C. metapsilosis* strains from different locations, however, reinforced this view, as all of them were found to descend from the same hybridization event, and constituted a successful, widespread and pathogenic hybrid lineage (Pryszcz *et al.* 2015). In a later study comprising 29 different strains of *C. orthopsilosis*, it was shown that most (93%) of the strains were hybrids, resulting from at least four independent hybridization events between the same two parental lineages (Schröder *et al.* 2016). In summary, the *C. parapsilosis* clade seems to have at least one fully homozygous lineage (*C. parapsilosis*), one lineage that is mostly formed by multiple, independently formed hybrids, although it contains some pure strains from *C. orthopsilosis*, and one lineage for which only hybrids are known (*C. metapsilosis*). Of note, the taxonomic description of this latter species is based on a hybrid strain. More recently, *Candida inconspicua*, another species from the CTG clade but distantly related to *C. parapsilosis*, was also found to constitute a hybrid, pathogenic lineage (Mixão *et al.* 2019). Altogether, these cases

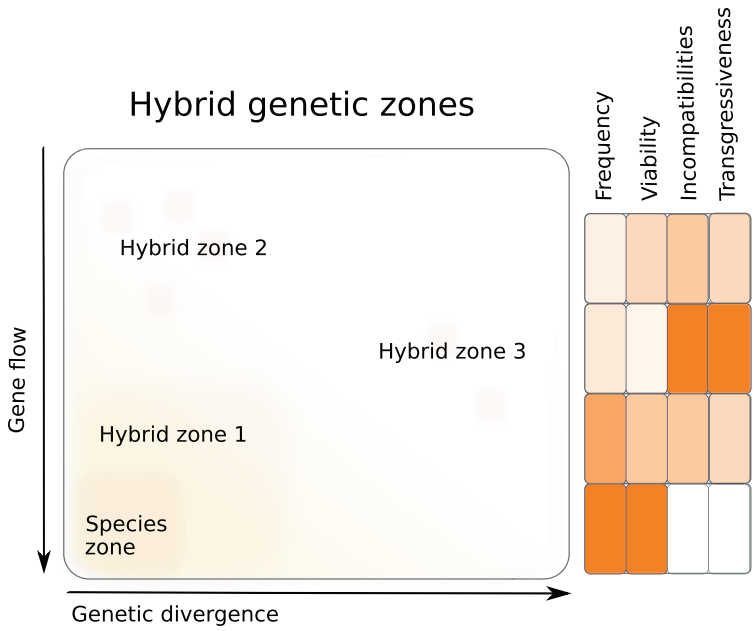


**Figure 2.** Hybrid genetic zones. Schematic representation of different types of hybrids depending on the level of genetic divergence and of gene flow between the hybridizing populations. The large square indicates a space of genetic relatedness (*x*-axis, genetic divergence) and connectivity (*y*-axis, amount of gene flow) between putative populations. The grid on the right indicates relative levels (darker color indicating higher levels) of frequency of formation of hybrids, the expected viability of the hybrids, the amount of genetic incompatibilities carried by these hybrids and the likelihood of presenting unique, transgressive phenotypes. These levels correspond, from top to bottom, to hybrids of zone 3, 2, 1 and the species zone, respectively. The species zone is defined by fuzzy boundaries in an area of low genetic divergence and high gene flow between populations. In this area, intra-species hybrids can be commonly found, which present high viabilities and low genetic incompatibilities. Some hybrids are able to back-cross with one of their parent lineages, which leads to introgression if it happens recurrently (i.e. the hybrid character remains only in small portions of the genome). The hybrid zone 1 is defined at areas where either gene flow or genetic relatedness is beyond the boundaries that usually define a species so that those populations rarely cross and often present some genetic incompatibilities. These hybrids are sometimes selected in very specific environments, and the same hybridization can independently be formed in similar environments. The sum of the species zone and the hybrid zone can sometimes correspond to what is commonly known as 'species complex' if hybrids are commonly found. Hybrids of zone 2 correspond to hybridization between populations that abruptly separated relatively recently, so that genetic divergence is still low, but the absence of gene flow between the populations may have resulted in the appearance of incompatibilities. Hybrids of zone 3 are hybrids between divergent lineages that are rarely formed and that are expected to present numerous incompatibilities. However, they can sometimes present unique, transgressive phenotypes that promote their survival in very specific niches.

represent mounting evidence that hybridization has promoted adaptation to the human host, and that hybrids may represent a significant fraction of the more rare yeast pathogens (Mixão and Gabaldón 2018). In this regard, analysis of the genomic patterns found in the highly heterozygous *C. albicans* is also suggestive of a hybrid past (Mixão and Gabaldón 2020) although in this case this would represent a more ancient hybridization. This would indicate that pathogenic hybrids might not be short lived, but could evolve by further adapting to its host. In fact, of the many *Candida* species that are able to infect humans (Gabaldón, Naranjo-Ortíz and Marcet-Houben 2016), *C. albicans* is the most adapted to the human host, being the one most commonly found as commensal, and also the primary source of candidiasis (Pfaller and Diekema 2007).

## THE GENOMIC AFTERMATH OF HYBRIDIZATION

The examples presented above suggest that hybridization is a rather common phenomenon in yeasts. This implies that hybrids not only do form often, but also that, in certain niches, they are selected over their non-hybrid parents. Hybrid organisms are chimeric from the start, harboring two divergent sets of chromosomes within a single nucleus. Most theoretical evolutionary frameworks would suggest this is a recipe for problems, as many incompatibilities between the two subgenomes, and the transcripts and proteins that they encode, would be expected. One such theoretical framework is that put forward by Bateson, Dobzhansky and Muller (Dobzhansky 1934; Muller 1942; Bateson 2009), also known as the Dobzhansky–Muller or the BDM model. This model predicts incompatibilities resulting from negative epistatic interactions between interacting genes that have independent evolutionary histories. Two diverging populations may accumulate different mutations and when different alleles of the same or interacting genes are brought together in the same organism by hybridization, these new combinations of genetic variability are expected to be less fit than combinations from alleles present in the same population, simply because they have never passed the filter of selection. Although new combinations of alleles may also give rise to fitness advantages in a given niche, these are likely to be a minority. It is expected that selection will purge negative epistatic interactions, while favoring the retention of the few advantageous ones.

Several processes can shape hybrid genomes (Fig. 3), including the duplication or loss of entire chromosomes or large chromosomal regions leading to chromosomal aneuploidies, gene loss, gene conversion or whole-genome duplication (Hirakawa *et al.* 2015; Marcet-Houben and Gabaldón 2015; Wolfe 2015; Albalat and Cañestro 2016; Dion-Côté and Barbash 2017; Smukowski Heil *et al.* 2017). Many of these processes contribute in one form or the other to progressive loss of heterozygosity (LOH), which are expected to promote genome stabilization by reducing the amount of incompatibilities. In fact loss

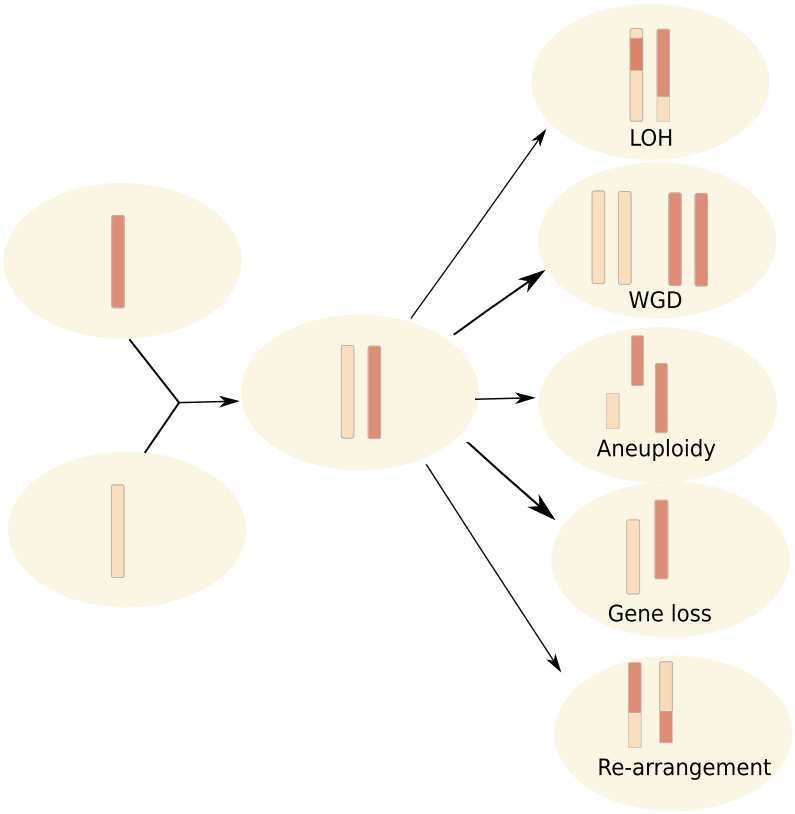


**Figure 3.** The genomic aftermath of hybridization. Schematic view of the main genomic processes following hybridization. When two cells belonging to diverged lineages cross (left), a hybrid is formed (center) in which the two chromosomal sets (here represented as a single chromosome with different colors) present the level of genetic divergence between the hybridizing parents. Processes such as, from top to bottom in the right part of the figure, loss of heterozygosity (LOH), genome duplication (WGD), appearance of aneuploidies, gene loss and genome re-arrangements can spontaneously appear, and subsequently selected if they eliminate or compensate negative epistatic interactions between the two subgenomes.

of heterozygosity (LOH) in regions that encode heterozygous genes involved in negative epistatic interactions is likely to be favored by natural selection (Mixão and Gabaldón 2018; Runemark, Vallejo-Marin and Meier 2019). Most yeast hybrids analyzed so far have been shown to present different levels of LOH in their genomes. However, it is still unclear to what extent these different levels of LOH reflect different elapsed times since the origin of the hybrids, varying rates of LOH or different degrees of selective pressure. In fact, the mechanisms and rates determining the observed patterns of LOH in the different hybrids are rather poorly characterized.

One particular case of aftermath of hybridization is the occurrence of a whole-genome duplication (WGD), or polyploidization, that is, the doubling of the entire genetic complement of an organism (Ohno 1970). Genome doubling can be achieved in two fundamentally different ways. In one, autopolyploidization, the replication of the genome is not followed by cell division, giving rise to a cell with essentially two identical copies of each chromosome. In another mechanism, allopolyploidization, the fusion of two distinct nuclei that is not followed by meiosis also results in a cell with a doubled genetic complement, but in this case each individual copy of the genomic complement is a different version, initially as divergent as the genomes of the two fused cells. If the two genomes are sufficiently divergent, as in inter-species hybridization, imperfect pairing of chromosomes may result in the inability to undergo meiosis. However, proper pairing among chromosomes can be restored upon further doubling of this genetic complement-polyploid with respect to the two haploid genomes that form the zygote but behaving as a pseudohaploid genome as it cannot reduce ploidy through meiosis. This process has been found to be common in plant evolution (Soltis and Soltis 2009; Soltis *et al.* 2015), and we have proposed as the mechanism leading to the yeast WGD (Marcet-Houben and Gabaldón 2015). More recently, this process has been found to have occurred in natural hybrids of *Zygosaccharomyces* (Ortiz-Merino *et al.* 2017; Watanabe *et al.* 2017; Braun-Galleani *et al.* 2018) and artificial hybrids of *Saccharomyces* (Charron *et al.* 2019), suggesting this might be a common mechanism for the restoration of fertility in hybrid lineages. In some cases, these duplications are predated by the damaging of one of the mat loci, a phenomenon that is also sometimes observed in allodiploid hybrids (Pryszcz *et al.* 2015; Mixão *et al.* 2019). Finally, the presence of polyploidies and aneuploidies is common in recent yeast hybrids (Querol and Bond 2009; Ortiz-Merino *et al.* 2018; Mixão *et al.* 2019), suggesting that allopolyploidization might not be a rare mechanism of hybridization. It is possible that, similar to the case of plants, ancient polyploidizations have driven the origin of other fungal lineages and that the rapid reshuffling of fungal chromosomes might render them difficult to detect (Campbell *et al.* 2016).

## THE TRANSCRIPTOMIC AFTERMATH OF HYBRIDIZATION

One particular case of potential incompatibilities between the two subgenomes of a hybrid is that of possible interference between the two regulatory networks. In this context, hybridization has been proposed to result in a so-called 'genomic shock' (McClintock 1984), characterized by massive disregulation of genes. The consequences of hybridization at the transcriptional level have been the focus of intense research in plants and animals (Ranz *et al.* 2004; Malone, Chrzanowski and Michalak 2007; McManus *et al.* 2010; Maheshwari and Barbash 2011). Most such studies reported a large impact of hybridization on the patterns of gene expression. In fungi, however, the few performed studies seem to suggest that the transcriptional shock elicited by hybridization might be rather limited. Transcriptomic studies in natural hybrids of the plant pathogen *Epichloë* Lp1 (Cox *et al.* 2014) and two independently formed hybrids of the opportunistic pathogen *Candida orthopsilosis* (Hovhannisyan *et al.* 2020a) revealed that the hybrid subgenomes retained gene expression levels similar to that in their homozygous parentals. In addition, the expression differences between homologous genes tended to be lower than the corresponding differences between the orthologous genes in the parental species, suggesting that hybridization buffers, rather than exacerbates transcriptional differences. However, as these studies are based on natural hybrids, they cannot disentangle whether the buffering of expression differences was achieved due to amelioration through compensatory mutations in the hybrid. The ease of manipulation of yeasts in the *Saccharomyces* genus allows artificial creation of hybrids, thereby enabling following the transcriptional aftermath from the start. This approach has been used to disentangle *cis* and *trans* effects in the evolution of gene regulation (Metzger, Wittkopp and Coolon 2017), the effect of different speed of the cellular cycle in the misexpression of genes (Swain Lenz, Riles and Fay 2014) or the effect of mitochondrial inheritance in the expression of hybrid nuclear genes (Hewitt *et al.* 2020). We recently directly asked the question of the impact of hybridization on gene expression, by investigating the extent of transcriptional shock in a newly formed *Saccharomyces cerevisiae* × *Saccharomyces uvarum* diploid hybrid and its diploid parentals (Hovhannisyan *et al.* 2020b). Despite the high divergence of the hybridizing species (~20 million years and around 25% at the nucleotide level), gene expression changes resulting from hybridization were very limited, affecting only ~1–2% of the genes. Comparatively, a thermal shock performed in the same study altered six times more genes. Overall, these results suggest that, in contrast to animals and plants, hybridization in yeasts may not be associated with a strong genomic shock at the transcriptional level. The underlying reasons for this remain unknown, but the inherent ability to buffer regulatory interferences between distant genomes may be related to the high frequency of hybridization in fungi. Although the overall changes in expression might be of a limited nature, the expression changes that indeed occur might have important functional consequences for the hybrid phenotype. An illustrating example is provided by the analysis of the maltotriose utilization in the lager beer yeast hybrid *S. pastorianus,* which resulted from the regulatory interaction between *S. cerevisiae* maltose transcription activator and the promoter of the *S. eubayanus* gene (Brouwers *et al.* 2019).

## CONCLUDING REMARKS

The advent of genomic technologies coupled to the power of comparative genomics and phylogenomics is unveiling processes that were previously invisible to us. Rather than being a rare phenomenon, hybridization seems to be common in fungi. In addition, and in contrast to the situation in animals and plants, fungal hybridization can cross large phylogenetic distances, producing chimeric organisms that defy our assumptions about the potential effects of epistatic interactions between alleles. Yeasts of the Saccharomycotina clade seem particularly prone to hybridization, with many examples of recently formed hybrids that are now thriving in industrial environments or are spreading as opportunistic pathogens. As these environments are the most intensively studied, it is likely that hybrids might be also common in some natural environments, particularly those with extreme conditions. Additionally, the formation of new hybrids might be promoted by human-related factors such as global trade, landscape alteration or global warming. Regarding this, the possible contribution of hybridization to the emergence of new pathogenic lineages is particularly worrying. More ancient hybrids have been recognized too, including the model pathogen *Candida albicans*, and the ancestor of the post-WGD clade of yeasts. This shows that hybrids are not necessarily a dead end of evolution, but that they can form new lineages that further diversify. How often has this happened is an open question, as ancient hybridization is definitely difficult to identify. We are only starting to have a glimpse of the genomic, transcriptomic and ecological aftermaths of hybridization, and further research is needed to better understand these processes.

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***Conflicts of Interest.*** None declared.

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