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,
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 hybrids were actually hybrids (Dujon 2012; Morales and Dujon 2012; Hittinger 2013; Leduq et al. 2016). More recently, the advent of genome sequencing and its broad use among Saccharomyces 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 (Przyszcz et al. 2014, 2015; Schröder et al. 2016; Mixão and Gabalón 2018; Mixão et al. 2019). Furthermore, yeast hybrids have also been identified in natural environments (Leduq et al. 2016). All these cases suggest that hybridization is a powerful driver for adaptation to novel environments. Indeed, the fact that most described Saccharomyces 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, Saccharomyces 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 Saccharomyces, 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 association studies recognized the hybrid nature of a Saccharomyces 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 Saccharomycocytina (Fig. 1), including Metschnikowia (Piombo et al. 2018), Zygosaccharomyces (Solieri, Cassanelli and Giudici 2007), Dekker (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 Saccharomycocytina 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 Saccharomycocytina, 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
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
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-Ortiz 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; Albatal 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
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 polyploidy and aneuploidy 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 level similar to that in their homozygous parents. 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, Wittkop 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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REFERENCES

Abbott R, Albach D, Ansell S et al. Hybridization and speciation. J Evol Biol 2013;26:229–46.
Albalat R, Cañestro C. Evolution by gene loss. Nat Rev Genet 2016;17:379–91.
Alsammar H, Delneri D. An update on the diversity, ecology and biogeography of the Saccharomyces genus. FEMS Yeast Res 2020;20:foa013.
Bateson W. Heredity and variation in modern lights. In: Seward AC (ed). Darwin and Modern Science. Cambridge: Cambridge University Press, 2009, 85–101.
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REFERENCES

Abbott R, Albach D, Ansell S et al. Hybridization and speciation. J Evol Biol 2013;26:229–46.
Albalat R, Cañestro C. Evolution by gene loss. Nat Rev Genet 2016;17:379–91.
Alsammar H, Delneri D. An update on the diversity, ecology and biogeography of the Saccharomyces genus. FEMS Yeast Res 2020;20:foa013.
Bateson W. Heredity and variation in modern lights. In: Seward AC (ed). Darwin and Modern Science. Cambridge: Cambridge University Press, 2009, 85–101.
Borneman AR, Zeppel R, Chambers PJ et al. Insights into the Dekkera bruxellensis genomic landscape: comparative genomics reveals variations in ploidy and nutrient
utilisation potential amongst wine isolates. PLoS Genet 2014;10:e1004161.

Braun-Galleani S, Ul Ortiz-Merino RA, Wu Q et al. Zygosaccharomyces pseudabailii, another yeast interspecies hybrid that regained fertility by damaging one of itsMAT loci. FEMS Yeast Res 2018;18:79.

Brouwers N, Brickwedde A, Gorter de Vries AR et al. Himalayan Saccharomyces eubayanus genome sequences reveal genetic markers explaining heterotic malto-riose consumption by Saccharomyces pastorianus hybrids. Appl Environ Microbiol 2019;85, DOI: 10.1128/AEM.01516-19.

Campbell MA, Ganley ARD, Gabaldón T et al. The case of the missing ancient fungal polyploids. Am Nat 2016;188:602–14.

Charron G, Marsit S, Hénault M et al. Spontaneous whole-genome duplication restores fertility in interspecific hybrids. Nat Commun 2019;10:1–10.

Choo JH, Hong CP, Lim JY et al. Whole-genome de novo sequencing, combined with RNA-Seq analysis, reveals unique genome and physiological features of the amylolytic yeast Saccharomyces fibuligera and its interspecies hybrid. Biotechnol Biofuels 2016;9:246.

Cox MP, Dong T, Shen GG et al. An interspecific fungal hybrid reveals cross-kingdom rules for allopolyploid gene expression patterns. PLoS Genet 2014;10:e1004180.

Crow JF. Advantages of sexual reproduction. Dev Genet 1994;15:205–13.

Curtin CD, Borneman AR, Chambers PJ et al. De-novo assembly of the heterozygous triploid genome of the wine spoilage yeast dekkera bruxellensis AWRI1499. PLoS One 2012;7:e33840.

Dion-Côté AM, Barbash DA. Beyond speciation genes: an overview of genome stability in evolution and speciation. Curr Opin Genet Dev 2017;47:17–23.

Dobzhansky T. Studies on hybrid sterility – I. Spermatogenesis in pure and hybrid Drosophila pseudoobscura. Zeitschrift für Zellforsch und Mikroskopische Anat 1934;21:169–223.

Dujon B. Yeast evolutionary genomics. In: Horst Feldmann (ed.). Yeast: Molecular and Cell Biology, Hoboken, New Jersey, United States. 2nd edn. 2012, 407–19.

Gabaldón T, Naranjo-Ortiz MA, Marcet-Houben M. Evolutionary genomics of yeast pathogens in the Saccharomyces. FEMS Yeast Res 2016;16:fow064.

Gallone B, Steensels J, Mertens S et al. Interspecific hybridization facilitates niche adaptation in beer yeast. Nat Ecol Evol 2019;3:1562–75.

Gibson B, Geertman J-MA, Hittinger CT et al. New yeasts-new brews: modern approaches to brewing yeast design and development. FEMS Yeast Res 2017;17, DOI: 10.1093/femsyr/fox038.

Hellborg L, Piskur J. Complex nature of the genome in a wine spoilage yeast, Dekkera bruxellensis. Eukaryot Cell 2009;8: 1739–49.

Hewitt SK, Duangrattanalert K, Burgis T et al. Plasticity of mitochondrial DNA inheritance and its impact on nuclear gene transcription in yeast hybrids. Microorganisms 2020;8:494.

Hirakawa MP, Martinez DA, Sakhthikumar S et al. Genetic and phenotypic intra-species variation in Candida albicans. Genomes Res 2015;25:413–25.

Hittinger CT. Saccharomyces diversity and evolution: a budding model genus. Trends Genet 2013;29:309–17.

Hovhannisyan H, Saus E, Ksiezopolska E et al. Integrative omics analysis reveals a limited transcriptional shock after yeast inter-species hybridization. Front Genet 2020b;11:404.

Hovhannisyan H, Saus E, Ksiezopolska E et al. The transcriptional aftermath in two independently formed hybrids of the opportunistic pathogen Candida orthopsilosis. mSphere 2020a;5, DOI: 10.1128/msphere.00282-20.

James SA, Bond CJ, Stratford M et al. Molecular evidence for the existence of natural hybrids in the genus Zygosaccharomyces. FEMS Yeast Res 2005;5:747–55.

Koumandou VL, Wickstead B, Ginger ML et al. Molecular paleontology and complexity in the last eukaryotic common ancestor. Crit Rev Biochem Mol Biol 2013;48:373–96.

Langdon QK, Peris D, Baker ECP et al. Fermentation innovation through complex hybridization of wild and domesticated yeasts. Nat Ecol Evol 2019;3:1576–86.

Leduci J-B, Nielly-Thibault L, Charron G et al. Speciation driven by hybridization and chromosomal plasticity in a wild yeast. Nat Microbiol 2016;1:15003.

Libkind D, Hittinger CT, Valério E et al. Microbe domestica- tion and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci USA 2011;108:14539–44.

Luis VL, Despons L, Friedrich A et al. Pichia sorbitophila, an interspecies yeast hybrid, reveals early steps of genome resolution after polyploidization. G3 Genomes, Genet, Genet 2012;2:299–311.

Madden AA, Epps MJ, Fukami T et al. The ecology of insect – yeast relationships and its relevance to human industry. Proc R Soc B Biol Sci 2018;285:20172733.

Maheshwari S, Barbash DA. The genetics of hybrid incompatibil- ities. Annu Rev Genet 2011;45:331–55.

Malone JH, Chrzanowski TH, Michalak P. Sterility and gene expression in hybrid males of xenopus laevis and X. muelleri. PLoS One 2007;2:e781.

Marcet-Houben M, Gabaldón T. Beyond the whole-genome duplication: phylogenetic evidence for an ancient inter-species hybridization in the baker’s yeast lineage. PLoS Biol 2015;13:e1002220.

McClintock B. The significance of responses of the genome to challenge. Science 1984;226:792–801.

McManus CJ, Coolon JD, Duff MO et al. Regulatory divergence in Drosophila revealed by mRNA-seq. Genome Res 2010;20:816–25.

Mendel G. Versuche Über Pflanzen-Hybriden. Brünn: Im Verlage des Vereines, 1866.

Metzger BPH, Wittkopp PJ, Coolon JD. Evolutionary dynamics of regulatory changes underlying gene expression divergence among Saccharomyces species. Genome Biol Evol 2017;9:843–54.

Mixão V, Gabaldón T. Genomic evidence for a hybrid origin of the yeast opportunistic pathogen Candida albicans. BMC Biol 2020;18:48.

Mixão V, Gabaldón T. Hybridization and emergence of virulence in opportunistic human yeast pathogens. Yeast 2018;35: 5–20.

Mixão V, Hansen AP, Saus E et al. Whole-genome sequencing of the opportunistic yeast pathogen candida inconspicua uncovers its hybrid origin. Front Genet 2019;10:383.

Morales L, Dujon B. Evolutionary role of interspecies hybridiza- tion and genetic exchanges in yeasts. Microbiol Mol Biol Rev 2012;76:721–39.

Müller HJ. Isolating mechanisms, evolution, and temperature. In: Dobzhansky T (ed). Biological Symposia: A Series of Volumes to Current Symposia in the Field of Biology. Lancaster, PA: Jaques Cattell Press, 1942;6:71–125.
Naranjo-Ortiz MA, Gabaldón T. Fungal evolution: cellular, genomic and metabolic complexity. Biol Rev 2020, DOI: 10.1111/brv.12605.

Ohno S. Evolution by Gene Duplication. Heidelberg: Springer-Verlag, 1970.

Ortiz-Merino RA, Kuanyshev N, Braun-Galleani S et al. Evolutionary restoration of fertility in an interspecies hybrid yeast, by whole-genome duplication after a failed mating-type switch. PLoS Biol 2017;15:e2002128.

Ortiz-Merino RA, Varela JA, Coughlan AY et al. Ploidy variation in Kluyveromyces marxianus separates dairy and non-dairy isolates. Front Genet 2018;9:94.

Peris D, Pérez-Torrado R, Hittinger CT et al. On the origins and industrial applications of Saccharomyces cerevisiae × S. Paradoxus adapted to olive brine. Front Genet 2019;10:449.

Pryszzcz LP, Nemeth T, Gacser A et al. Genome comparison of Candida orthopsilosis clinical strains reveals the existence of hybrids between two distinct subspecies. Genome Biol Evol 2014;6:1069–78.

Pryszzcz LP, Németh T, Saus E et al. The genomic aftermath of hybridization in the opportunist pathogen Candida metapsilosis. PLoS Genet 2015;11:e1005626.

Querol A, Bond U. The complex and dynamic genomes of industrial yeasts: MINIREVIEW. FEMS Microbiol Lett 2009;293:1–10.

Ranz JM, Namgyal K, Gibson G et al. Anomalies in the expression profile of interspecific hybrids of Drosophila melanogaster and Drosophila simulans. Genome Res 2004;14:373–9.

Runemark A, Vallejo-Marin M, Meier J. Eukaryote hybrid genomes. PLoS Genet 2019;15:e1008404.

Schröder MS, Martinez de San Vicente K, Prandini THR et al. Multiple origins of the pathogenic yeast Candida orthopsilosis by separate hybridizations between two parental species. PLoS Genet 2016;12:e1006404.

Shen XX, Opulente DA, Kominek J et al. Tempo and mode of genome evolution in the budding yeast subphylum. Cell 2018;175:1533–45. e20.

Smukowski Heil C, Burton JN, Liachko I et al. Identification of a novel interspecific hybrid yeast from a metagenomic spontaneously inoculated beer sample using Hi-C. Yeast 2018;35:71–84.

Smukowski Heil CS, DeSevo CG, Pai DA et al. Loss of heterozygosity drives adaptation in hybrid yeast. Mol Biol Evol 2017;34:1596–612.

Soltis PS, Marchant DB, Van de Peer Y et al. Polyploidy and genome evolution in plants. Curr Opin Genet Dev 2015;35:119–25.

Soltis PS, Soltis DE. The role of hybridization in plant speciation. Annu Rev Plant Biol 2009;60:561–88.

Stefanini I, Dapporto I, Berti I et al. Social wasps are a saccharomyces mating nest. Proc Natl Acad Sci USA 2016;113:2247–51.

Swain Lenz D, Riles L, Fay JC. Heterochronic meiotic mis-expression in an interspecific yeast hybrid. Mol Biol Evol 2014;31:1333–42.

Tavanti A, Davidson AD, Gow NAR et al. Candida orthopsilosis and Candida metapsilosis spp. nov. to replace Candida parapsilosis groups II and III. J Clin Microbiol 2005;43:284–92.

Varela JA, Puricelli M, Ortiz-Merino RA et al. Origin of lactose fermentation in Kluyveromyces lactis by interspecies transfer of a neo-functionalized gene cluster during domestication. Curr Biol 2019;29:4284–90. e2.

Vaughan Martini A, Martini A. Three newly delimited species of Saccharomyces sensu stricto. Antonie Van Leeuwenhoek 1987;53:77–84.

Walther A, Hesselbart A, Wendland J. Genome sequence of Saccharomyces carlsbergensis, the world’s first pure culture lager yeast. G3 Genes, Genomes, Genet 2014;4:1–11.

Watanabe J, Uehara K, Mogi Y et al. Mechanism for restoration of fertility in hybrid Zygosaccharomyces rouxii generated by interspecies hybridization. Appl Environ Microbiol 2017;83: e01187–17.

Wolfe KH. Origin of the yeast whole-genome duplication. PLoS Biol 2015;13:e1002221.