Saccharomyces yeast hybrids on the rise

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Funding information
 Wenner-Gren Foundations, Grant/Award Numbers: UPD2018-0196, UPD2019-0110; Stockholm University, Grant/Award Number: SU FV-1.2.1-0124-17; Knut and Alice Wallenberg Foundation, Grant/Award Number: 2017.0163; Swedish Research Council, Grant/Award Number: 2017-04963

Abstract
Saccharomyces hybrid yeasts are receiving increasing attention as a powerful model system to understand adaptation to environmental stress and speciation mechanisms, using experimental evolution and omics techniques. We compiled all genomic resources available from public repositories of the eight recognized Saccharomyces species and their interspecific hybrids. We present the newest numbers on genomes sequenced, assemblies, annotations, and sequencing runs, and an updated species phylogeny using orthogroup inference. While genomic resources are highly skewed towards Saccharomyces cerevisiae, there is a noticeable movement to use wild, recently discovered yeast species in recent years. To illustrate the degree and potential causes of reproductive isolation, we reanalyzed published data on hybrid spore viabilities across the entire genus and tested for the role of genetic, geographic, and ecological divergence within and between species (28 cross types and 371 independent crosses). Hybrid viability generally decreased with parental genetic distance likely due to antirecombination and negative epistasis, but notable exceptions emphasize the importance of strain-specific structural variation and ploidy differences. Surprisingly, the viability of crosses within species varied widely, from near reproductive isolation to near-perfect viability. Geographic and ecological origins of the parents predicted cross viability to an extent, but with certain caveats. Finally, we highlight publication trends in the field and point out areas of special interest, where hybrid yeasts are particularly promising for innovation through research and development, and experimental evolution and fermentation.

Take Away
This article provides (1) a quantitative review of all genomic resources of Saccharomyces yeast species and their hybrids, (2) a compilation of published data on reproductive isolation (spore viabilities) within and between species, (3) highlights and trends in the publication efforts within this field, and (4) areas where yeast hybrids are and will be particularly useful for research and development in the future.

Keywords
experimental evolution, fermentation, genomics, hybridization, reproductive isolation, spore viability, yeast
1 | INTRODUCTION

While botanists had acknowledged the evolutionary importance of hybrids over a century ago (Stebbins, 1959), it took modern sequencing technology to convince a broad audience that hybrids are ubiquitous and that genetic exchange between divergent lineages can indeed generate biodiversity. Hybridization and other interspecific sources of novel genomic variation such as horizontal gene transfer (HGT) have played important roles in the adaptive evolution of many plant and animal groups (Abbott et al., 2013; Arnold & Arnold, 2006; Mallet, 2007). Hybridization also substantially contributed to generating the diversity of microbial fungal species we see today (Dujon & Louis, 2017; Taylor & Larson, 2019). Our understanding of the evolutionary outcomes of hybridization in the yeast genus Saccharomyces is constantly growing and includes genomic, physiological, and ecological impacts of hybridization (Alsammar & Delneri, 2020; Bendixsen, Peris, et al., 2021; Brice et al., 2021; Gabaldón, 2020; Lancaster et al., 2019; Pérez-Través et al., 2014; Smukowski Heil et al., 2019).

Species in the yeast genus Saccharomyces are of particular interest because they hybridize readily not only in the laboratory but also in natural and domesticated environments despite significant genetic and ecological differences. This makes them a powerful model to study the role of hybridization in adaptive evolution (Botstein & Fink, 2011; Hittinger, 2013). The impact of hybridization on the evolutionary history of Saccharomyces has been discussed in a number of recent reviews (Alsammar & Delneri, 2020; Boynton & Greig, 2014; Gabaldón, 2020; Gladieux et al., 2014; Morales & Dujon, 2012; Ono et al., 2020). But this is a fast-moving field that has seen the discovery of five new Saccharomyces species in the last two decades alone, with new wild strains and interspecific and intraspecific hybrids discovered every year thanks to fast and affordable genome sequencing. Here, we provide an update of the recent research efforts generating (1) new genomic resources of all eight recognized Saccharomyces species and their hybrids; (2) data on the degree of reproductive isolation between species pairs as a function of genetic, geographic, and ecological differences; and (3) publication trends in this field over the last three decades. We start by reconstructing the phylogeny of the eight currently recognized Saccharomyces species, using the most up-to-date high-quality genome assemblies. We provide summary statistics on the number of sequenced genomes, assemblies, annotations, and sequencing runs available per species. We then provide a comprehensive overview of all genomic resources available to date on interspecific Saccharomyces hybrids. Next, we review and reanalyze available data on the viability of intraspecific and interspecific crosses to provide an overview of the patterns, distributions, and possible drivers of reproductive isolation in this species group. To emphasize the substantial research efforts occurring in this field, we also quantified the number of publications on Saccharomyces yeasts and their hybrids over the last three decades, parsed by species affiliations (see Box 1). Lastly, we highlight two fields where interest in Saccharomyces hybrids is on the rise: experimental evolution and beverage fermentation.

2 | GENOMIC RESOURCES FOR SACCHAROMYCES

Members of the monophyletic clade Saccharomyces (formerly Saccharomyces sensu stricto, Figure 1) are referred to as budding yeast owing to their life cycle. The genus contains a large range of evolutionary distances, from recently separated populations (differentiated by only a few single nucleotide polymorphisms [SNPs]) to geographically and genetically distant populations within species (1%–4% nucleotide divergence; Bendixsen, Gettle, et al., 2021; Liti et al., 2009; Ono et al., 2020) and eight currently recognized, vastly divergent species (10%–22% nucleotide divergence; Figures 1 and S8; Brice et al., 2021). Genetic divergence is thought to be largely driven by geographic isolation resulting in varying degrees of reproductive isolation (Liti et al., 2009; Marsit et al., 2017). For decades, Saccharomyces species were defined according to the biological species concept suggesting members within a species are reproductively compatible and members of different species are reproductively isolated (Liti et al., 2006; Ono et al., 2020). Although this is often true, as we discuss below, the lines between species within this genus are often blurred by hybridization. At the same time, crosses between strains belonging to the same species can have extremely low spore viabilities. In recent years, congruent to advances in sequencing technologies, genetic differences have been relied upon to establish taxonomy, species boundaries, and evolutionary histories (Bendixsen, Gettle, et al., 2021; Scannell et al., 2011). One of the most fascinating insights from concerted efforts in comparative genomics is the evidence of an ancient whole-genome duplication (WGD) event at the onset of the Saccharomyces radiation (Dujon et al., 2004; Kellis et al., 2004; Wolfe et al., 1997), which is suggested to be a consequence of hybridization (Marcet-Houben & Gabaldón, 2015; Wolfe, 2015) and served to provide stability and fertility of the new hybrid lineages (Charron et al., 2019). Evidence for genomic admixture also comes from mitochondrial introgressions suggesting extensive ancestral hybridization among Saccharomyces species (Peris, Arias, et al., 2017). Thus, hybridization is strongly woven into the origin and natural history of the Saccharomyces yeast diversity we see today.

To give context to the significance and rise of hybrids within this clade, we built a phylogenetic tree containing the eight currently recognized species (Saccharomyces cerevisiae, Saccharomyces paradoxus, Saccharomyces mikatae, Saccharomyces jurei, Saccharomyces kudriavzevii, Saccharomyces arboricola, Saccharomyces eubayanus, and Saccharomyces uvarum) using the most up-to-date high-quality genomic assemblies (Figure 1). The tree was built by orthogroup inference using OrthoFinder (Emms & Kelly, 2015, 2019) and species tree inference using STRIDE (Emms & Kelly, 2017) and STAG (Emms & Kelly, 2018). Notably, there are three sister species pairs (S. cerevisiae–S. paradoxus, S. mikatae–S. jurei, and S. eubayanus–S. uvarum) which share evolutionary histories with more recent divergence. There are also several strains within the genus that were previously thought to be pure species but are now recognized as historical hybrids (e.g., S. pastorianus and S. bayanus, not shown in phylogeny). The sequencing of over 3000 Saccharomyces genomes, in over 50,000
sequencing runs, resulting in over 1000 assemblies and over 240,000 annotations, has led to a wealth of genomic insight (Figure 1). The number of genomes sequenced was found in a recent review (Libkind et al., 2020), and the number of assemblies, annotated sequences, and sequencing runs was obtained from the European Nucleotide Archive (ENA). Not surprisingly, the vast majority of these resources (76% of genomes sequenced and 87% of genome assemblies) are within *S. cerevisiae*. Other species, such as *S. paradoxus*, *S. eubayanus*, and *S. uvarum*, have received moderate attention (12%, 9%, and 2% of total genomes sequenced, respectively). The other species (*S. mikatae*, *S. jurei*, *S. kudriavzevii*, and *S. arboricola*) have very limited genomic resources (<1%). These disparities are likely a result of the recent discovery of these species, their geographically confined distribution and seemingly limited diversity, and their perceived usefulness for industrial applications (but see Hutzler et al., 2021).

3 | NEW GENOMIC RESOURCES FOR SACCHAROMYCES HYBRIDS

The genus *Saccharomyces* has been described as an evolutionary paradox (Ono & Greig, 2019). Species in this genus are genomically extremely divergent (10%-20% nucleotide differences genome-wide, which is on the scale of humans and mice; Shen et al., 2018) but are able to generate fertile and viable hybrid offspring at low frequency. Interspecific hybrids have been found in the wild and estimates suggest that ~10% of all yeast isolates and to date ~6% of sequenced genomes are hybrids (Libkind et al., 2020; Liti et al., 2006). Some strains seem to have persisted for hundreds to tens of thousands of years. Recently, a living ancestor of an ancient hybridization event was isolated that still retained the ancestral genome structure of the first-generation hybrid (D’Angiolo et al., 2020). But most sequenced *Saccharomyces* hybrids are used in biotechnology, food and beverage production, and also increasingly as research model organisms to investigate evolutionary processes with experimental evolution, time-series sampling, and comparative genomics (see Sections 5.1 and 5.2).

To date, 242 hybrid genomes have been sequenced, the bulk of them (175) in the last 3 years (Table S1). Notably, this list has been extensively expanded recently due to two novel investigations into interspecific hybrid yeast in fermentation environments (Gallone et al., 2019; Langdon et al., 2019). Unfortunately, it is often difficult to differentiate between natural and more recent lab-generated hybrids. For this review, we have made no distinction between the two. Some hybrid genomes (~7%) have more than two major genomic contributors, but most have two parent species. Synthetic hybrids have been constructed with genomic contributions from up to six different yeast species (Peris et al., 2020). The sequenced hybrids are not uniformly distributed among all possible pairwise species combinations.

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**FIGURE 1** Phylogenetic relationships of *Saccharomyces* species. The tree was built by orthogroup inference using OrthoFinder and species tree inference using STRIDE and STAG. Support values are the proportion of times that the bipartition is seen in each of the individual species tree estimates. Branch lengths represent the average number of substitutions per site across the sampled gene families. Representatives from each species were selected, and annotated genomes were obtained from published studies and publicly accessible data (Baker et al., 2015; Liti et al., 2009, 2013; Naseeb et al., 2018; Scannell et al., 2011). *Kluyveromyces lactis* and *Torulaspora delbrueckii* were used as outgroups but are not shown in the tree. The number of genomes sequenced per species was found in a recent review (Libkind et al., 2020). The number of assemblies, annotated sequences, and sequencing runs for each species was obtained from the European Nucleotide Archive. Counts are colored on a log-normalized scale for each category (white–orange–red) [Colour figure can be viewed at wileyonlinelibrary.com]
Of the 28 two-species combinations, only six combinations have available genomes, and these are dominated by *S. cerevisiae* × *S. eubayanus* crosses (47%), many of which are closely related to each other and very similar in their genomic composition (Bendixsen, Peris, et al., 2021). *S. cerevisiae* × *S. eubayanus* hybrids, also known as *S. pastorianus*, are the most common interspecific hybrids used for brewing. *S. pastorianus* was generated accidentally in beer and wine fermentation environments in the 15th century, combining the cold tolerance and ethanol resistance of its parents (Dunn & Sherlock, 2008; Libkind et al., 2011). The unique history and genomic origin of this historical hybrid has been discussed elsewhere (Bing et al., 2014; Boynton & Greig, 2014; Libkind et al., 2011).

An overwhelming proportion (~86%) of all sequenced hybrids has genomic contributions from *S. cerevisiae*. This species bias in sequencing is linked to environmental preferences (Figure 2b); most hybrids (~79%) were isolated from warm, nutrient-rich fermentation environments (~65% lager/beer, ~14% wine, and ~0.4% whiskey), where *S. cerevisiae* is the dominant species. This bias towards *S. cerevisiae* might also be partially attributed to standard laboratory techniques, such as culturing conditions, having been highly optimized for *S. cerevisiae*. One interesting exception is the number of “relic” *S. cerevisiae* × *S. paradoxus* hybrid genomes isolated from olive brine (Pontes et al., 2019) making up ~9% of all available genomes. The remaining 12% of hybrids were collected in wild, fruit, unknown, clinical, laboratory, baking, or dietary environments. We anticipate a significant expansion of the currently limited genomic resources for hybrids in the near future. A fruitful strategy to isolate more hybrid strains from the wild may be to target environments with extreme or fluctuating conditions (e.g., temperature, carbon source, or pH), such as insect guts (Madden et al., 2022; Stefanini, 2018; Sung-Oui et al., 2005), which may be conducive for hybrid yeasts with novel resistance and metabolic phenotypes. The high prevalence of aneuploidies found in interspecific hybrids may further confer adaptation to extreme environments (outlined in Gilchrist & Stelkens, 2019). Besides the genomes clearly identified as hybrid strains, introgressed genetic material from donor to recipient species is also frequently found in sequencing studies, ranging widely in size and type. Introgression is common in domesticated species (Almeida et al., 2014; Peter et al., 2018) but has also been found in wild populations (Almeida et al., 2017; Liti et al., 2006), affecting both the nuclear and mitochondrial genome (Peris, Arias, et al., 2017). Introgression from *S. paradoxus* into *S. cerevisiae* is particularly common with up to hundreds of open reading frames (ORF) identified (Bendixsen, Peris, et al., 2021; Peter et al., 2018). In fact, all *S. cerevisiae* isolates have been shown to carry at least one *S. paradoxus* gene, suggesting pervasive gene-flow between the two species (Peter et al., 2018). Introgression is used as genetic evidence for past hybrid matings followed by backcrossing and other postzygotic processes, causing an imbalance in the relative contributions of the parental genomes. Bendixsen, Peris, et al., (2021) recently identified a relationship between parental contributions and aneuploidy in hybrids using read depth analysis: The larger the difference in parental genomic content in the hybrid genome, the fewer aneuploidies were detected, which is likely a result of increasing genome stabilization after hybridization event.

**FIGURE 2** Sequenced genomes of interspecific *Saccharomyces* hybrids. (a) The number of sequenced genomes available for each interspecific hybrid cross is indicated at the intersection of the two parental species. *Saccharomyces cerevisiae* × *Saccharomyces eubayanus* hybrids include known historic hybrid *Saccharomyces pastorianus*. Node size and color indicate the number of genomes. “Complex” indicates interspecific hybrids with significant genomic contributions from more than two species. (b) The environmental origins of the sequenced hybrid genomes. “Other” includes all environments contributing less than 2% (unknown, wild, clinical, laboratory, baking, dietary, and whiskey) [Colour figure can be viewed at wileyonlinelibrary.com]
Science Core Collection was searched for publications containing *Saccharomyces*, hybrid and the species name in the “topic” (searching title, abstract, and keywords) in the last three decades (1991–2019). For species with more than one name, species synonyms were included. Publications including Zygosaccharomyces, yeast two-hybrid, or yeast microarray were removed as false hits. For this analysis, we did not differentiate among articles, reviews, and other types of publications and included publications on both interspecific and intraspecific hybrids. Members of *Saccharomyces* were the subject of over 5000 research publications per year for the last decade. Publications with hybrids averaged ~150 publications per year (Figure 3). These studies are largely dominated by the pillar species, *S. cerevisiae*. The other species contributed only 2% to the total number of publications over the last three decades. Entering the 21st century, research on *Saccharomyces* hybrids peaked at 217 publications a year and slowly decreased for the following decade. These publications were dominated by hybrids with at least one *S. cerevisiae* parent (Figure 3). Recent years have seen a resurgence in hybrid yeast research. In particular, studies on hybrids with non-*cerevisiae* parents have increased within the last 6 years, from 4.4 to 9.6 to almost 40 publications per year over the last three decades. This increase is mainly attributed to a heightened interest in studying and using novel hybrid genomes in experimental evolution and beverage fermentation (see Section 5).

4 | REPRODUCTIVE ISOLATION IN SACCHAROMYCES

Genome evolution produces genetic divergence and reduces interbreeding between nascent lineages; however, the relationship between sequence divergence and reproductive isolation among yeast species remains unclear (Ono et al., 2020; Ono & Greig, 2019). The *Saccharomyces* life cycle consists of both asexual and sexual stages. Cells in the wild usually exist as diploids, reproducing vegetatively by mitosis. Under stress, especially nitrogen starvation, cells undergo meiosis, which produces an ascus containing four haploid spores. When haploid spores of opposite mating types from divergent intraspecific or interspecific strains are in close proximity, hybridization can occur, generating a heterozygous F1 that contains chromosomal copies from both parents. F1 hybrids generated from distantly related strains and species are generally less likely to produce viable spores than closely related strains, mainly due to faulty chromosomal segregation in hybrid meiosis (Bozdag et al., 2021, see Box 2 for a short review of postzygotic isolation mechanism in *Saccharomyces*).

Hybrid viability is often strain- and cross-specific and can vary widely. A comprehensive analysis of hybrid viability, including all species combinations and multiple strains within species, is lacking so far. Here, to quantify the degree of divergence and infertility of both intraspecific and interspecific crosses, we compiled and reanalyzed data available from the literature on F1 spore viability (i.e., recombined haploid F2 spores) for each of the eight *Saccharomyces* species and their hybrids. In summary, we found data on 32 cross types (8 intraspecific and 24 interspecific) and 434 independent crosses (i.e., involving different strains and/or reported in different
Despite their vast evolutionary divergence spanning millions of years, all known Saccharomyces species have surprisingly collinear genomes. Hybrid sterility between Saccharomyces species is mainly caused by antirecombination, causing chromosomal missegregation in F1 hybrid meiosis. This renders the majority of F2 hybrids aneuploid and inviable (Bendixsen et al., 2021; Bozdag et al., 2021; Hunter et al., 1996; Rogers et al., 2018; Zhang et al., 2020). Other contributors to reproductive isolation are complex negative epistatic interactions involving multiple nuclear and mitochondrial loci (Chou et al., 2010; Hou et al., 2014; Lee et al., 2008), and mitochondrial-nuclear interactions significantly affect the QTL landscape of interspecific hybrids (Naseeb et al., 2021). Decreased spore viability with increasing parental genetic distance (Figure 5b) is consistent with antirecombination and negative epistasis becoming more frequent with increasing sequence divergence. Strain-specific deviations from this general pattern are likely due to differences in ploidy, large structural variation, and genomic reshuffling, but this seems to play a larger relative role in RI within species than between species (Stelkens & Greig, 2016).

Mechanisms leading to increased sequence homology between chromosomes improve segregation during meiosis and increases hybrid fertility. One such mechanism is WGD, which provides a perfectly homozygous partner chromosome for correct pairing during meiosis that then yields euploid gametes (Marsit et al., 2021; Naseeb et al., 2021). Another mechanism is return-to-growth (RTG), where meiosis is aborted after genome duplication and mitotic growth resumes with a diploid genome (Honigberg & Esposito, 1994), restoring fertility and allowing recombination in normally sterile crosses (Mozzachiodi et al., 2021). Another homology increasing process is loss-of-heterozygosity (LOH) caused by break-induced replication or gene conversion (Dayani et al., 2011; Laureau et al., 2016). LOH can rescue hybrid fertility in interspecific (Dutta et al., 2021) and interspecific hybrids (D’Angiolo et al., 2020) as the newly homozygous sequence blocks restore regular meiotic recombination. Smukowski Heil et al. (2017) found positive selection for gene-specific LOH events in S. cerevisiae × S. uvarum hybrids, and a follow-up study showed that the fitness effects of LOH are environment-dependent and more extreme in hybrids than in intraspecific crosses (Lancaster et al., 2019). Together with the fact that LOH occurs at a higher rate than mutation, this suggests a vital role for LOH in hybrid yeast genome evolution and adaptation to stressful environments.
When crosses involving lab strains are removed from the comparison, there is no longer any statistically significant effect of ecological origins ($t = 0.43; p = 0.67$). Crosses within *S. cerevisiae* range from 2.5% to 100% viability with a mean of /C24/75% (Bendixsen, Gettle, et al., 2021; Cubillos et al., 2011; Hou et al., 2014; Naumov et al., 1997; Naumov et al., 2001; Naumov et al., 2003; Naumov et al., 2013; Naumov, Masneuf, et al., 2000; Sniegowski et al., 2002). This large variation is surprising given the generally low level of genomic divergence found within *S. cerevisiae*, which does not exceed 1.4% (Bendixsen, Gettle, et al., 2021; Duan et al., 2018; Peter et al., 2018). Geographic region did not explain significant difference in spore viability within *S. cerevisiae* ($t = -1.27; p = 0.21$, Figure 4a). Most notably, strains isolated in North America exhibit relatively high viability with strains from all other regions (Figure S1), likely reflecting the rather recent introduction of wine strains to North America. On the other hand, *S. cerevisiae* strains differ considerably in their ecological preferences and are found in a vast diversity of habitats (soil, fruit, baking, brewing, and clinical environments to name just a few). This may suggest that ecological differences between strains are the primary cause for reproductive isolation within *S. cerevisiae*. We did find that the environmental origins of strains affected viability but, counterintuitively, we see higher spore viabilities in crosses between environments ($t = -2.85; p = 0.01$, Figure 4a). However, as discussed above, this trend is strongly driven by high viabilities among crosses involving lab strains (Figure S2). Alternatively, some of the *S. cerevisiae* strains included here may have large structural rearrangements (translocations, inversions, etc.), which disrupt chromosomal synteny and lead to low spore viability. For example, a strain isolated from Malaysia (UWOPS03-461.4) has been shown to have severely reshuffled chromosomes (Marie-Nelly et al., 2014; Yue et al., 2017) resulting in a high level of reproductive isolation with all other strains (Cubillos et al., 2011). Domestication within *S. cerevisiae* has also been
shown to significantly affect spore viability and is driven by aneuploidies and gene function losses (De Chiara et al., 2020).

*S. paradoxus* also displays a large range of intraspecific spore viabilities with some strains almost entirely reproductively isolated (<1% viability), while others maintain high spore viabilities of up to 95% (Liti et al., 2006; Naumov et al., 1997; Naumov et al., 2003; Naumov, James, et al., 2000; Naumov, Naumova. Hagler, et al., 1995; Sniegowski et al., 2002; Stelkens et al., 2014). We observed a slightly trimodal response, but most viabilities clustered around 50% (mean viability was 43%). This broad range of viabilities is likely attributed to the large genomic variation within *S. paradoxus* (~3.8%), untouched by domestication, and their vast geographic distribution (Boynton & Greig, 2014). Importantly, crosses from the same geographic region are significantly more viable (31% higher spore viability) than crosses from different regions (*t* = 4.07; *p* < 0.001, Figures 4a and S1). In particular, strains from South America are highly reproductively isolated from any other region but maintain high viability within the region. Ecological origin also contributed to spore viability within *S. paradoxus* (*t* = 2.48; *p* = 0.02). Crosses from the same ecological niche have on average ~20% higher viability compared to between niche crosses (Figure 4a).

*S. kudriavzevii* (26%–90%), *S. eubayanus* (18%–89%), and *S. uvarum* (22%–100%) also show large ranges of viabilities (Almeida et al., 2014; Bing et al., 2014; Hittinger et al., 2010; Naumov, 2000; Naumov et al., 2001; Naumov et al., 2003; Naumov, 1996; Naumov, James, et al., 2000). The other two species with low counts of available spore viability data, *S. jurei* (n = 1, Naseeb et al., 2017) and *S. arboricola* (n = 4, Naumov et al., 2013; Naumov et al., 2010), both have very high average interspecific cross viabilities at approximately 90%. We need more data from *S. mikatae*, *S. jurei*, and *S. arboricola* to be able to test the impact of geography and ecology on intraspecific cross viability.

### 4.2 | Interspecific hybrid viability

Most interspecific crosses show low spore viability (Figure 5a) as expected given the large genetic divergence between these species.

![Figure 5](https://example.com/figure5.png)
(10%–22% nucleotide differences genome-wide). However, in some crosses, especially between sister species (S. cerevisiae × S. paradoxus and S. eubayanus × S. uvarum), we found ~9% spore viability on average, albeit with a range of viabilities depending on which strains were crossed (Table S3). Many S. cerevisiae × S. paradoxus crosses result in 0% viable spores, but others surprisingly generate up to 65% viable spores (Table S3). Similarly, S. eubayanus × S. uvarum crosses range from 1% to 19% viable spores. We also found 13 species crosses where all available spore viabilities are 0 (Figure 5a). This complete absence of viability is likely due to using low-throughput tetrad dissection approaches with limited sample sizes. We were unable to find viability data for four species crosses, all involving S. eubayanus (Figure 5a). Given the large genetic divergence of these two species from all others in the genus (Figure 1), we expect these species pairs to have very low spore viability.

To test if genome-wide sequence divergence predicts reproductive isolation, we calculated genetic distances between all species pairs (Figure S3). As representatives of each species, we used the assembled genomes used for the phylogenetic tree in Figure 1 (Baker et al., 2015; Liti et al., 2009; Liti et al., 2013; Naseeb et al., 2018; Scannell et al., 2011). Genetic distances were calculated using estimated DNA–DNA hybridization values generated using the Genometo-Genome Distance Calculator 3.0 with formula 2 (Meier-Kolthoff et al., 2013; Meier-Kolthoff et al., 2021). This formula estimates distance by locally aligning the genomes using BLAST+ (Camacho et al., 2009), resulting in matches called high-scoring segment pairs which represent local alignments that are considered statistically significant. The sum of all identical base pairs over all significant local alignments is then divided by the total alignment lengths. As expected, the three pairs of sister species (S. cerevisiae–S. paradoxus, S. mikatae–S. jurei, and S. eubayanus–S. uvarum) have the lowest amount of genetic divergence due to their shared evolutionary histories (Figure S3).

We found a significant negative correlation between spore viability and genetic distance using linear regression ($r^2 = 0.53, p < 0.001$; Figure 5b). There were some notable deviations from this trend. Despite being the most recently diverged species pair and having the lowest genetic distance, S. mikatae × S. jurei crosses generated spores with very low viability (~1.5% on average). However, this mean was calculated from only two crosses and is not necessarily representative. Another interesting deviation was found in S. cerevisiae × S. kudriavzevi hybrids, which, despite their large genetic distance, have unexpectedly high mean spore viability (~2.8%; calculated from seven different crosses: five with low viability [0–0.83%] and two with high viability [>7%]). These patterns are in agreement with antirecombination and negative epistasis becoming more frequent at larger parental sequence divergence and strain-specific structural variation and ploidy differences playing a role in reproductive isolation between some strains as well.

Due to limited sample sizes and overall low viability for most interspecific hybrid crosses, it was difficult to test for relationships between spore viability and geographic or ecological origins (Figure S4). Some geographic regions (Asia and South America) show relatively high viability within the region (~9%), while European strains have lower average viability (~2%, Figure S5). We found a single significant interaction in S. cerevisiae × S. paradoxus hybrids where viability is higher between ecological origins, than within (Figure S4), but this is driven by a couple of high viability crosses (Figure S6 and Table S3). In summary, we found no substantial patterns between interspecific spore viability and ecological origin across all species (Figure S7) and in the most common species crosses (Figure S8).

5 | HARNESING THE POTENTIAL OF SACCHAROMYCES HYBRIDS

Given the vast genetic diversity within each Saccharomyces species and especially between species, there is immense potential for employing intraspecific and interspecific hybrids in research and industrial applications (Sipiczki, 2018). Two fields in particular show an increase in popularity, experimental evolution, and beverage fermentation. Here, we review the powerful resource that hybrid strains represent in these fields and highlight which interspecific hybrid crosses are most often employed.

5.1 | Experimental evolution approaches with hybrids

Saccharomyces hybrids have proven to be a powerful tool in studying and employing evolutionary processes. The last three decades have seen a stark rise in the number of experiments using Saccharomyces hybrids in evolutionary studies (Figure 6a). In the last decade, numbers have more than doubled, and publications have covered nine of the possible 28 interspecific crosses and four of eight crosses within species. The large majority (~91%) of the 161 publications in this decade had significant genomic contributions from S. cerevisiae. Recently, hybrid yeast have been employed in experimental evolution studies because they offer a novel approach to understand complex adaptive processes and dynamics, including but not limited to ecological speciation (Greig et al., 2002), LOH (Lancaster et al., 2019; Smukowski Heil et al., 2017), aneuploidy (Gorter de Vries et al., 2019; Zhang et al., 2020), transposon mobility and accumulation (Hénault et al., 2020; Smukowski Heil et al., 2021), whole-genome duplications (Charron et al., 2019; Marsit et al., 2021), and QTL analysis (Naseeb et al., 2021). Each of these fields has made major advancements in the past decade by methodically applying experimental evolution to Saccharomyces hybrids, allowing for novel insights into uncharted biological processes.

5.2 | Hybrid strains in beverage fermentation

Due to the vast amount of novel genetic combinations in hybrids, the potential for identifying a new phenotype that can be exploited for fermenting beverages is immense. The number of studies employing
Saccharomyces hybrids for fermentation has grown steadily and has risen sharply from five to eight per year in previous decades to ~22 per year (Figure 6b). Recent publications covered eight out of 28 possible interspecific crosses and four out of eight intraspecific crosses. Of the 236 publications in this decade, ~95% had significant genomic contributions from \textit{S. cerevisiae}, but there was a notable shift toward more \textit{S. cerevisiae} × \textit{S. kudriavzevii} hybrids and \textit{S. cerevisiae} × \textit{S. eubayanus} (\textit{S. pastorianus}) hybrids. These hybrid combinations have long histories of being used in fermenting wine and beer (Erny et al., 2012; González et al., 2006; Martini & Martini, 1987; Monerawela & Bond, 2018). The potential for new, novel combinations of genes (Hewitt et al., 2014; Nakao et al., 2009; Naseeb et al., 2021) and proteins (Piatkowska et al., 2013) has sparked a great interest in developing \textit{Saccharomyces} hybrids in industrial environments (Giannakou et al., 2021). The goal for these cultivated hybrids is to amplify the best features of each parent while minimizing parental limitations, to breed unique hybrid-specific phenotypes. An important limitation in \textit{S. cerevisiae} is tolerance to low temperature, which can be overcome by crossing with a more cryotolerant species such as \textit{S. eubayanus}, \textit{S. kudriavzevii}, or \textit{S. uvarum} (García-Ríos et al., 2019; Hebly et al., 2015; Krogerus et al., 2021). Given the consistent discovery and isolation of new strains within \textit{Saccharomyces}, we anticipate that hybridization will continue to play a vital role in beverage fermentation for the foreseeable future.

\textbf{FIGURE 6} Publication record for evolution and fermented beverages. (a) Number of publications with \textit{Saccharomyces} AND hybrid* AND evolution in the topic. Dots are colored according to the total number of publications in a given year (dark blue = maximum; white = minimum). Inset breaks down which specific hybrids were studied in the past decade (2010–2019). Interspecific hybrids are indicated at the intersection between two species. \textit{Saccharomyces cerevisiae} × \textit{Saccharomyces eubayanus} hybrids include known historic hybrid \textit{Saccharomyces pastorianus}. Intraspecific hybrids are found along the diagonal. The number of publications is indicated by the size and color of the dots as indicated in the legend. Missing dots indicate no publications. “Complex” indicates interspecific hybrids with significant genomic contribution from more than two species, including known historic hybrid \textit{Saccharomyces bayanus}. (b) Number of publications with \textit{Saccharomyces} AND hybrid* AND beer OR \textit{Saccharomyces} AND hybrid* AND wine in the topic. Dots are colored according to the total number of publications in a given year (dark red = maximum; white = minimum). Inset follows the same pattern as panel (a) [Colour figure can be viewed at wileyonlinelibrary.com]
FUTURE OUTLOOK FOR HYBRID YEAST RESEARCH

We expect more strains of the recently described, non-domesticated Saccharomyces species (S. mikatae, S. arboricola, and S. jurei) to be isolated from the wild and sequenced in the near future, advancing our understanding of their geographic distribution, phylogenetic history, demography, and ecological backgrounds. We also hope to see these species included in more studies on intraspecific and interspecific hybrid viability and fitness, helping us answer questions about the relative importance of different isolation and speciation mechanisms at work in this group, for example, whether different strain ecology, genome-wide sequence divergence, or large structural genomic differences contribute to reproductive isolation. Hybrid genomes assembled from long sequencing reads will be tremendously useful here as they will resolve aspects of genome architecture known to be important for hybrid fitness and adaptation, which have been difficult or even impossible to determine from short-read sequencing alone. For instance, long-read sequencing enables us to better identify large structural variations, assemble low complexity or repetitive regions such as transposable elements, and track regions of LOH and recombination. There is reason to believe that the shock of genome admixture at the early stages of hybrid speciation, leading to high aneuploidy and massive LOH events, has contributed to the evolutionary success of some hybrid lineages (outlined in Gilchrist & Stelkens, 2019). This is difficult to prove as aneuploidies are usually only an ephemeral feature of the genome. We need more reliable read depth data, temporal environmental sampling, and evolution experiments with aneuploid hybrids, to scrutinize the role of aneuploidy in hybrid stress resistance and adaptation to new niches.

Finally, humans have used Baker’s yeast for food production for millennia, but the vast majority of species are not adapted to high-throughput industrial processes. Yet recent work suggests that interspecific hybridization can facilitate adaptation and diversification into different niches during beer fermentation, making the process more efficient (Gallone et al., 2019) and the product more desirable (Langdon et al., 2019). This is good news considering there is an urgent need for more sustainable ways to produce energy, food, and medicine. Hybrids may provide achievable solutions and interesting innovations. We suggest that it is time to explore the genomes of more wild species, and their hybrids, representing a vast ecological reservoir of genes and phenotypes potentially useful for bioбережика, biocontrol, biofuel production, and other sustainable purposes (Libkind et al., 2020; Peris, Moriarty, et al., 2017). Engineering wildtype genes into industrial strains and using hybrids in experimental evolution for strain optimization are becoming vital strategies to tackle pressing sustainability issues.

ACKNOWLEDGMENTS

This work was supported by the Swedish Research Council (2017-04963 to RS), the Knut and Alice Wallenberg Foundation (2017.0163 to RS), the paired PhD program of the Faculty of Science, Stockholm University (SU FV-1.2.1-0124-17 to RS), and the Wenner-Gren Foundations (UPD2018-0196 and UPD2019-0110 to DPB). We are also grateful for help from Kristoffer Krogerus, Walter P. Pfleigler, and David Peris in identifying and providing hybrid spore viability data, and to Noah Gettle for comments on the manuscript.

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How to cite this article: Bendixsen, D. P., Frazão, J. G., & Stelkens, R. (2022). *Saccharomyces* yeast hybrids on the rise. *Yeast*, 39(1), 40–54. [https://doi.org/10.1002/yea.3684](https://doi.org/10.1002/yea.3684)