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Minireview

Visualizing the next frontiers in wine yeast research

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One sentence summary: This minireview envisages and explores the next frontiers in wine yeast research spurred by bioinformational engineering and synthetic genomics.
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Abstract

A range of game-changing biodigital and biodesign technologies are coming of age all around us, transforming our world in complex ways that are hard to predict. Not a day goes by without news of how data-centric engineering, algorithm-driven modelling, and biocyber technologies—including the convergence of artificial intelligence, machine learning, automated robotics, quantum computing, and genome editing—will change our world. If we are to be better at expecting the unexpected in the world of wine, we need to gain deeper insights into the potential and limitations of these technological developments and advances along with their promise and perils. This article anticipates how these fast-expanding bioinformational and biodesign toolkits might lead to the creation of synthetic organisms and model systems, and ultimately new understandings of biological complexities could be achieved. A total of four future frontiers in wine yeast research are discussed in this article: the construction of fully synthetic yeast genomes, including minimal genomes; supernumerary pan-genome neochromosomes; synthetic metagenomes; and synthetic yeast communities. These four concepts are at varying stages of development with plenty of technological pitfalls to overcome before such model chromosomes, genomes, strains, and yeast communities could illuminate some of the ill-understood aspects of yeast resilience, fermentation performance, flavour biosynthesis, and ecological interactions in vineyard and winery settings. From a winemaker's perspective, some of these ideas might be considered as far-fetched and, as such, tempting to ignore. However, synthetic biologists know that by exploring these futuristic concepts in the laboratory could well forge new research frontiers to deepen our understanding of the complexities of consistently producing fine wines with different fermentation processes from distinctive viticultural terroirs. As the saying goes in the disruptive technology industry, it take years to create an overnight success. The purpose of this article is neither to glorify any of these concepts as a panacea to all ills nor to crucify them as a danger to winemaking traditions. Rather, this article suggests that these proposed research endeavours deserve due consideration because they are likely to cast new light on the genetic blind spots of wine yeasts, and how they interact as communities in vineyards and wineries. Future-focussed research is, of course, designed to be subject to revision as new data and technologies become available. Successful dislodging of old paradigms with transformative innovations will require open-mindedness and pragmatism, not dogmatism—and this can make for a catch-22 situation in an archetypal traditional industry, such as the wine industry, with its rich territorial and socio-cultural connotations.

Keywords: minimal genome, pan-genome, *Saccharomyces cerevisiae*, supernumerary neochromosome, synthetic communities, synthetic genome, wine yeast

Global challenges and trends in an uncertain world

It has been a tough start to this decade. As we are now transitioning from a devastating pandemic to a manageable epidemic, an uncertain and unsettled world finds itself in a state of fragmentation, disequilibrium, contestation, and adaptation. Abundant shared global challenges are compounded in demographic, environmental, economic, and technological developments—inextricably intertwined with society dynamics and evolving geopolitical rivalries—which are shaping the contours of our future world (www.dni.gov/nic/globaltrends). The forces driving these emerging global challenges and trends include slowing population growth and rising median age; deepening inequality and cultural polarization; rapid migration and urbanization; intensifying physical effects of climate change, land degradation, and pollution; rising debt, fragmented trading, and employment disruptions; and the increasing pace, reach, and impact of technological developments. In particular, the blistering global race for technological dominance and supremacy is set to cause exponential change to the world as we know it.

The astonishingly rapid development and worldwide rollout of coronavirus vaccines in 2021 is an illustrative case. The increasing convergence of seemingly unrelated fields of research and the escalation of global competition to secure advantage in today's much more contested world are accelerating the emergence of cutting-edge technologies and the sparking of transformative innovations. Ideas that existed only on chalkboards a few decades ago, are now poised to disrupt major industries around the world. However, the ways in which ideas like mRNA-based vaccines morph into practical solutions remain unpredictable. Although the anti-Covid mRNA vaccines seemed to have been created almost instantly, the underpinning technology drew upon nearly five decades of basic research, shaped by unexpected technological difficulties as well as unanticipated breakthroughs in multiple fields.

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As we are contemplating what disruptive technologies might be about to burst into prominence and open up new frontiers and possibilities in the wine industry, it is worth reminding ourselves of the mantra that it takes many years of research, discovery and development to create an overnight success. Technological disruption does not just happen; it is a gradual process, which industries learn to adapt to through strategy and preparation. Disruption can come from anywhere. It is not just about the technology but also how it is deployed to improve existing products and generate new ones, enhance services, or deliver process efficiencies at scale. No industry is disruption-proof and it is, therefore, prudent to prepare for continual disruption in an environment where uncertainty is the only certainty. Even in science, there is often not absolute certainty. Multidisciplinary research around well-considered theories and hypotheses is the best way to reduce the level of uncertainty. The discovery path to absolute truth is a science of uncertainty and an art of probability—the so-called *uncertainty paradox*, which is an umbrella term for situations in which uncertainty is acknowledged, but the role of science is framed.

The purpose of this article is to acknowledge the complex uncertainties of our times while anticipating which technological areas appear to offer the potential for transformative change in the wine industry over the next couple of decades. More specifically, it is proposed that the next frontiers in wine yeast research might be spurred by the convergence of bioinformational engineering and biodesign technologies (Dixon et al. 2020, 2021a,b). A total of four specific future-focussed research concepts are explored in this article: the construction of fully synthetic yeast genomes, including minimal genomes; supernumerary pan-genome neochromosomes; synthetic metagenomes; and synthetic yeast communities. This minireview accepts that the proposed research will encounter multiple twists and turns and that there are no guarantees for impactful practical outcomes. However, it is hoped that research around these four concepts will illuminate some of the intriguing dark secrets of how wine yeasts interact individually and collectively in vineyards and wineries. As we are exploring the unknown in wine research, we will have to tolerate the uncertainties and continue to be fascinated by the probabilities.

Predictable uncertainty in winemaking

Winemakers know all about uncertainty. Thanks to the unpredictability of weather events and seasonal changes, winemakers are rolling the dice with Mother Nature every vintage. The success of each particular vintage depends on that year's weather conditions, especially temperature and the amount of sunshine and water (and when in the life cycle of the vines the water arrived). As a seasonal agricultural product, the natural quality of grapes and the wine produced from them varies from year to year. As a result of yearly variation, the concept of *vintage* is unique to wine. Unlike other food products' *best-before date*, the quality of wine is linked to *vintage*, and—in some wine categories—also to *terroir* (Pretorius 2020). The importance of *vintage variation* in the marketplace reflects the fact that *fine wines* (especially reds) are not consumed immediately because they require a period of maturation or ageing.

Modern trends in the grapegrowing sector produce grapes from a whole gamut of cultivation systems—from fully fledged biodynamic and organic grapegrowing to large-scale mechanized viticulture where weed killing, pesticides, fertilizers, and irrigation are used to even out the vagaries of the growing conditions as much as possible from vintage to vintage (Vivier and Pretorius 2002). Viticulturists often take starkly divergent approaches to address similar challenges in the vineyard. If done appropriately, all of these viticultural approaches can produce high-quality grapes and good-quality wine. Biodynamic viticulture (Reeve et al. 2005) probably represents an extreme form of *vintage variation* in an individual vineyard, whereas mechanized viticulture produces wine with more *consistent* quality across vintages that was not previously available at this level of predictability and scale. Generally, small-scale grapegrowers at the 'cottage end' of the industry tend to turn to organic viticulture while large producers take more advantage of the latest technologies and practices available in viticulture.

These general trends in viticulture are mirrored in vinification. Boutique wineries tend to rely on the *wild yeasts* present on grape skins to spontaneously ferment the must. These winemakers embrace the concept *terroir* as part of their *winegrowing* practices and embed *vintage variation* at the heart of their marketing strategy (Pretorius 2020). They accept the risks that, in some years, the quality of their wines might be compromised due to prolonged fermentation times or the presence of spoilage microbes. They are willing to roll the dice in the marketplace where consumers might rave about the *complexity* and *roundness* of their wines in some years and not in others. At the other end of the spectrum, large-scale producers prefer to inoculate their grape must with reliable *Saccharomyces cerevisiae* wine yeast starter cultures and selected strains of *Oenococcus oeni* malolactic bacteria, thereby mitigating potential risks of sluggish/stuck fermentations and wine spoilage by the presence of undesirable microbes originating from the vineyard or winery equipment (Pretorius 2000). Large-scale wineries normally aim to smooth out vintage variation, thereby striving for predictable quality outcomes and consistency of their *clean*, *fault-free* wines across vintages. These vintners try to anticipate potential shifts of consumer preferences in their target market segments. They are also willing to adopt new technologies and to adapt their practices across the entire supply chain (Fig. 1) so that they can shape their wines to predetermined specifications, thereby meeting the latest preferences of their targeted consumers (Pretorius and Bauer 2002, Pretorius and Høj 2005).

This article rejects the false dichotomy that *fine wine* can only be made by either the traditional practice of *spontaneous* fermentation (also known as *uninoculated* or *natural* ferments) or by the more contemporary practice of *inoculated* fermentation (Swiegers et al. 2005, Pretorius 2016). Rather, this article peeks over the far horizons of future technological developments stemming from the convergence of bioinformational engineering and synthetic genomics. As new frontiers in yeast research are being forged with these technologies, fresh ideas and future-shaping trends are emerging around fully synthetic yeast chromosomes, genomes, and communities.

The following sections present a vision of how these futuristic synthetic systems can be developed, explored, and used to deepen our fundamental understanding of wine yeasts and population dynamics in model systems, and how such learnings could ultimately bring potential benefits to practical winemaking.

Synthetic yeast genomes rich with scientific and oenological marvels

It is estimated that the speciation process in the *Saccharomyces* yeast clade commenced with a whole genome duplication (WGD) event dating back ~100 million years ago (Belda et al. 2019). Over the eons, *Saccharomyces* evolved as a fierce microbial competitor in sugar-rich environments (e.g. ripening fruits) by developing an

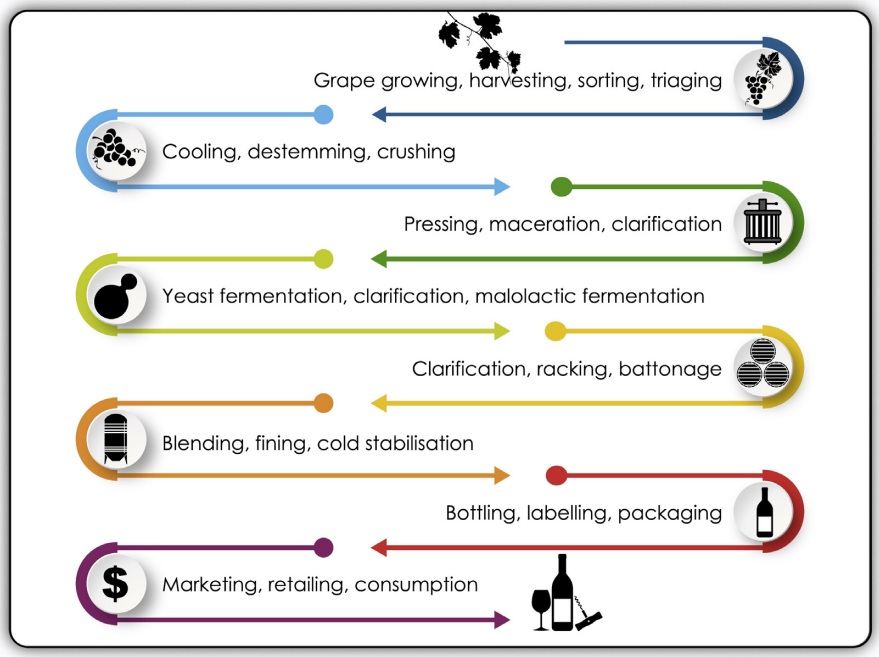

Figure 1. The basic process of winemaking. The *from-grapes-to-glass* chain of steps are subject to the forces of *market-pull* and *technology-push*, which often spark tension between *tradition* and *innovation*.

ability to make, accumulate, tolerate, and consume ethanol as an energy source (Goddard and Greig 2015, Marsit and Dequin 2015, Gallone et al. 2016, Steensels et al. 2019). This competitive strategy arose from genomic changes that increased the flux from hexose to ethanol and enabled the consumption of ethanol (e.g. duplication of *ADH1* and *ADH2*), as well as promoter rewiring resulting in the loss of regulatory elements from genes involved in respiration (Liti 2015).

The competitiveness of this unicellular 'sugar fungus' was further honed at the end of the Sone Age when humans transitioned from their hunting–gathering lifestyle to farming during the Neolithic Revolution (the First Agricultural Revolution) about 12 000 years ago (Chambers and Pretorius 2010, Steensels et al. 2019, Dixon and Pretorius 2020). As humankind switched from a nomadic to sedentary society, the process of yeast fermentation was discovered, harnessed, and improved in terms of process control and reproducibility; food safety; and smell and taste (Steensels et al. 2019). The natural selection that occurred during various anthropogenic domestication events, as well as the more recent human interventions, moulded the genomes of *Saccharomyces* species for a diverse range of niche fermentation processes for the production of safe and tasty food and alcoholic beverages (Bergström et al. 2014, Borneman et al. 2016, Legras et al. 2018, Peter et al. 2018; Fig. 2). For example, bakers, brewers, distillers, and winemakers started to select strains and natural variants of *S. cerevisiae* that best met their needs (Borneman et al. 2008, 2011, 2012, 2013a,b, 2015, Hyma et al. 2011, Almeida et al. 2015, Pretorius 2017a, Steensels et al. 2019).

It was not until the 20th century that geneticists took a research interest in this fascinating food-grade, budding yeast. It was first observed microscopically by Antonie van Leeuwenhoek in 1676, and implicated as a living agent, which is able to convert grape must into wine, by Louis Pasteur in 1859 (Pretorius 2000, Jagtap et al. 2017, Dixon and Pretorius 2020). Genetic research started in earnest with the pioneering work of Øjvind Winge (1930s), Carl and Gertrude Lindegren (1940s and 1950s), Robert Mortimer (1960s and 1970s), and their collaborators, which eventuated in the development of the first comprehensive chromosomal maps (1970s and 1980s) of the reference haploid *S. cerevisiae* strain, S288C (Dixon and Pretorius 2020). The final chromosomal map showed that the genome of this haploid strain comprised 16 linear chromosomes, varying from ~200 to ~2000 kb. Having such a comprehensive chromosomal map, sparked the interest of molecular biologists to utilize this genetically mapped eukaryote as an experimental model for gene cloning (from the late 1970s) and genome sequencing (early 1990s). In 1996, an international consortium of 94 laboratories from 19 countries announced the first complete genome sequence of *S. cerevisiae* (Goffeau et al. 1996, Oliver 1996). Various sequencing methods and technologies were used by the different laboratories. Most of the sequencing was done with two isogenic strains of S288C named AB972 (ATCC 76269) and FY (ATCC 96604; Engel et al. 2014). So, the genome sequence of *S. cerevisiae* published in 1996, is that of the reference haploid lab strain S288C (Goffeau et al. 1996, Oliver 1996). The maintenance and annotation of this genome sequence are provided by the *Saccharomyces* Genome Database (SGD; www.syntheticyeast.org), one of the original model organism databases (Engel et al. 2014). The S288C genome sequence was resequenced and updated in 2010. The updated version, called 'S288C 2010', was determined from a single yeast colony using modern sequencing technologies and now serves as the reference *S. cerevisiae* genome sequence for ongoing innovations in yeast genomic research (Engel et al. 2014, Pretorius 2017b; Pretorius and Boeke 2018).

So far, the updated reference *S. cerevisiae* genome sequence revealed the following information. The total genome size of *S. cerevisiae* S288C includes 12.07 Mb of chromosomal DNA, 85 kb of mitochondrial DNA, and 6.3-kb episomal plasmids (2 μ; Pretorius and Boeke 2018; Belda et al. 2019). The genome contains 6604 open reading frames (ORFs) with 79% of the ORFs verified, 11%

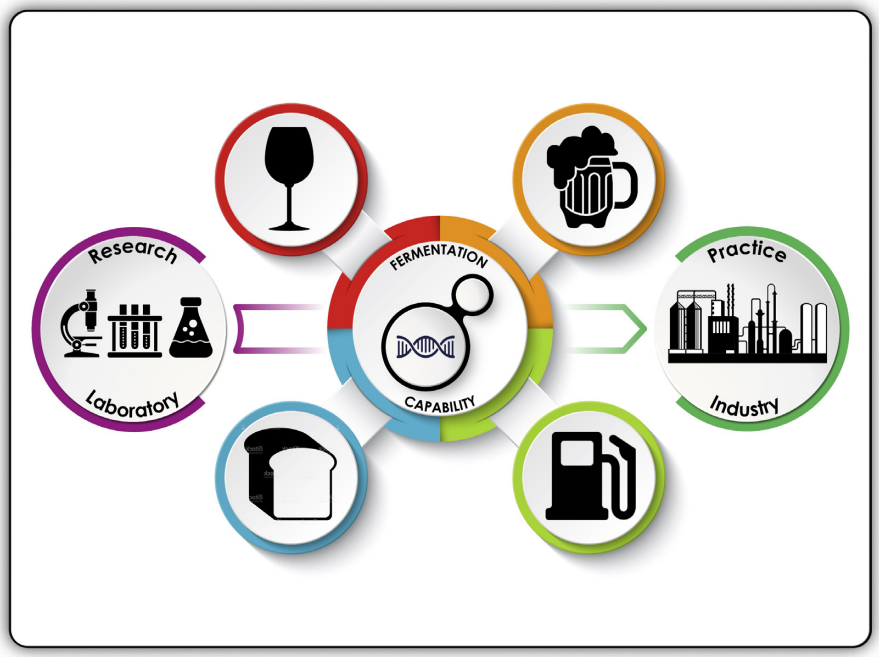

Figure 2. *Saccharomyces cerevisiae* is a versatile yeast with a rich fermentation history. *Saccharomyces cerevisiae* developed a so-called Crabtree-positive carbon metabolism as a highly efficient strategy for sugar utilization that enables energy generation under fermentative or anaerobic conditions and restricts the growth of competing microorganisms by producing toxic metabolites, such as ethanol and carbon dioxide. This yeast species is not only the preeminent model eukaryotic model organism for research but also the most widely used microbe in the biofuel, food, and alcoholic beverage industries, and more recently in the pharmaceutical and biotechnological industries.

uncharacterized, and 10% regarded as dubious, with 1786 of the ORFs still annotated to unknown function (Belda et al. 2019). The genome carries 428 RNA genes (299 tRNA, 77 snoRNA, 27 rRNA, 18 ncRNA, and six snRNA), one telomerase RNA, 295 introns in 280 genes with nine genes containing more than one intron (Engel et al. 2014; Pretorius and Boeke 2018). At least 55 genes found in other 'well-studied' *S. cerevisiae* genomes are absent in S288C. There are more than 500 sets of paralogs (www.yeastgenome.org).

The early availability of a well-curated reference genome for *S. cerevisiae*—i.e. being updated and annotated on an ongoing basis by the SGD—made this industrially important yeast with 'generally regarded as safe' (GRAS) status the leading and best-studied eukaryotic model organism (Pretorius et al. 2012). The insights gained through the genomic analysis of this supermodel reference yeast positioned *S. cerevisiae* S288C well for crossing the frontier into the territory of synthetic genomics. With the announcement of the first draft synthetic set of its 16 linear chromosomes, this yeast strain, Sc2.0, will become the first eukaryote with a computer-designed, man-made genome (Richardson et al. 2017; Pretorius and Boeke 2018). Currently, these 16 draft synthetic chromosomes are being 'growth-defect debugged' before they will be consolidated into a single Sc2.0 yeast cell. The ultimate Sc2.0 strain will contain a streamlined (removal of transposons and nonessential introns) and defragmented (relocation of all tRNA genes to a separate, supernumerary mini-chromosome) genome with standardized telomeres, a 'freed-up' TGA stop codon, PCR-Tags recognition labels and multiple LoxPsym sites (Fig. 3).

The synthetic model genome of the Sc2.0 strain will not only reveal fundamental biological knowledge but will also enable synthetic chromosome recombination by LoxP-mediated evolution, SCRaMbLE. The induction of SCRaMbLE leads to inversions, duplications, translocations, and deletions of genes flanked by LoxPsym sites, thereby generating numerous genomes with unique gene content and genome architecture (Shen et al. 2016, 2018, Blount et al. 2018, Hochrein et al. 2018, Jia et al. 2018, Liu et al. 2018, Wu et al. 2018, Luo et al. 2018b). The application of SCRaMbLE together with 'Clustered Regularly Interspaced Short Palindromic Repeats' (CRISPR) genome editing technologies offer tremendous opportunity for karyotype engineering and prototype strain development. Ideally, the application of SCRaMbLE and CRISPR to the Sc2.0 genome will enable the generation of extremely high levels of genetic variation and simultaneous incorporation of foreign DNA and new non-native yeast phenotypes. Such a scenario will open new vistas for yeast strain development and genome minimization.

The synthesis of minimal genomes will not only facilitate deeper understanding of fundamental genome biology but will also remove some of the biological complexities that currently hinder the introduction of a larger array of oenologically important traits. One of the lessons learnt while constructing the Sc2.0 synthetic chromosomes is that despite the variety of changes introduced, yeast cells are quite tolerant to these perturbations. Based on the plasticity of the Sc2.0 genome, it has been proposed to introduce more radical changes to generate a much more compact Sc3.0 genome (Dai et al. 2020). The design and synthesis of the proposed Sc3.0 reduced genome rely on the completion of the Sc2.0 genome. It is envisaged to restructure all essential genes from each of the 16 chromosomes with designated regulatory elements. The next step is to functionally validate and assemble each of these chromosomes into a dedicated chromosome with altered gene orders. The final step is then to combine the Sc3.0 chromosomes in a single cell to obtain strains with multiple chromosomes (Mitchell et al. 2017), or alternatively, to merge these Sc3.0 chromosomes into a single large chromosome (Shao et al. 2018, Luo et al. 2018a). Complementary to Sc2.0, the Sc3.0 genome could reveal (i) how much of the yeast genome is redundant and to what

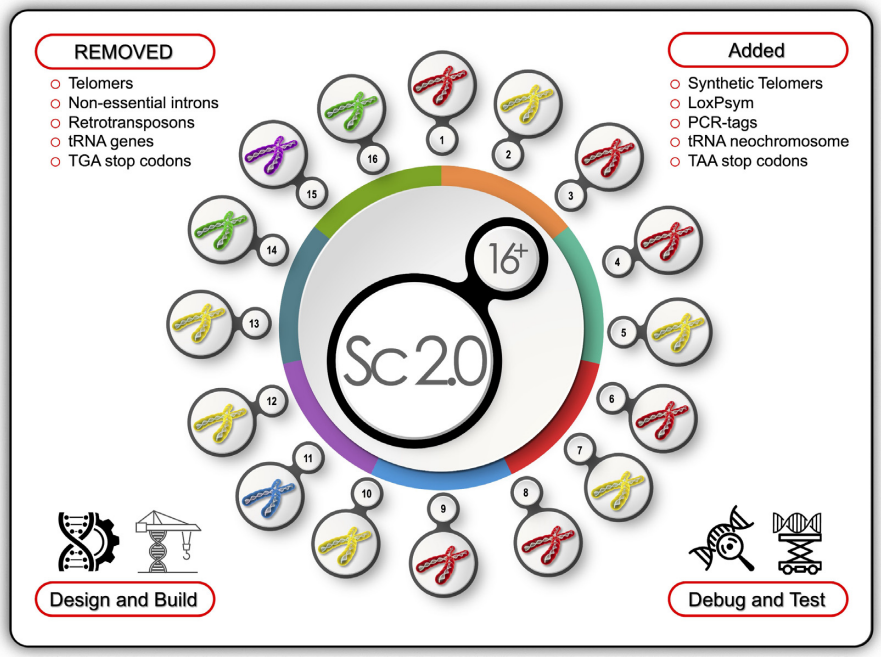

Figure 3. The design and construction of a synthetic *S. cerevisiae* S288C genome. The Sc2.0 genome is designed to encode a slightly modified genetic code in which (i) all 16 chromosomes contain the same synthetic telomeres; (ii) all nonessential introns and transposons are removed; (iii) all tRNA genes are translocated to a 17th mini-chromosome; (iv) all TGA stop codons are recoded to TAA; (v) PCR-tags and LoxPsym sites are added; and (vi) inconvenient restriction enzyme sites are removed.

extent it could be compacted; (ii) what is the content of a minimal genome to support life under a given condition; and (iii) whether the gene organization in the natural *S. cerevisiae* genome is evolutionary inevitable or contingent (Dai et al. 2020). Many challenges will have to be overcome. For example, (i) misregulation of any essential gene could lead to inviable cells; (ii) altering most DNA sequences can cause long-range interactions to be disrupted, potentially leading to dysfunction; and (iii) it could be difficult to coordinate the expression of co-regulated genes using synthetic regulatory elements (Dai et al. 2020).

Tailor-made synthetic genomes and customized minimal genomes for different industrial settings have the potential to design purpose-built wine strains at unprecedented scale.

Pan-genome neochromosomes endowed with phenotypic diversity

The laboratory-bred S288C strain used for building the synthetic Sc2.0 strain, will be used for the construction of the Sc3.0 minimal genome, lacks many genes that provide phenotypic diversity to the hundreds of environmental and industrial isolates of *S. cerevisiae*. These isolates exhibit distinctive phenotypes, many of which provide selective advantage within specific environmental niches or industries, such as the wine industry. Such phenotypic differences are the direct result of intraspecific genetic variation, often in the form of strain-specific genes of gene clusters (Hall and Dietrich 2007; Liti et al. 2009, Novo et al. 2009, Akao et al. 2011, Borneman et al. 2011, 2016, Borneman and Pretorius 2015, Peter et al. 2018). The differential presence of these genes and gene clusters among *S. cerevisiae* strains can impart important phenotypic traits, which are absent in S288C. By comparing the genome sequences of these environmental and industrial strains to that of the S288C reference strain, it becomes obvious that S288C seems to represent an almost minimal core set of genes shared with environmental and industrial *S. cerevisiae* strains (Fig. 4).

To address the lack of the pan-genomic ORFs and the missing genetic variation in S288C, and to provide for additional phenotypic plasticity in the Sc2.0 parental strain, a synthetic new-to-nature chromosome was engineered (Kutyna et al. 2022). This supernumerary neochromosome contains a diverse spectrum of pan-genomic elements, usually found in environmental and industrial *S. cerevisiae* strains. The design of this neochromosome is compatible with the Sc2.0 design. Inclusion of the neochromosome as a 17th chromosome was shown to provide phenotypic plasticity to the Sc2.0 strain, including expanding the range of utilizable carbon sources (Kutyna et al. 2022). Further adaptive gains were made by the induction of programmable structural variation. This suggests that the presence of this pan-genome neochromosome in the Sc2.0 strain could pave the way for the adaptation of Sc2.0 to a wider variety of environments. This would be a way to transition the Sc2.0-derived synthetic strains from laboratory into industrial applications (Kutyna et al. 2022).

Synthetic metagenomes encapsulating ecosystems in a cell

The complexity of life in different viticultural terroirs and spontaneous wine ferments goes beyond a single species. Naturally, grape-associated yeasts live in microbial communities with each microbe occupying a niche, i.e. essential for functioning of the ecosystem in a vineyard. It is important to understand all components of a vineyard's ecology as an intricate metabolic network. Community-level metabolic network modelling could resolve the ecological properties of vineyard microbiomes and even identify keystone species in the maintenance of ecosystem phenotypes within a particular terroir or vineyard (Muller et al. 2018). Such

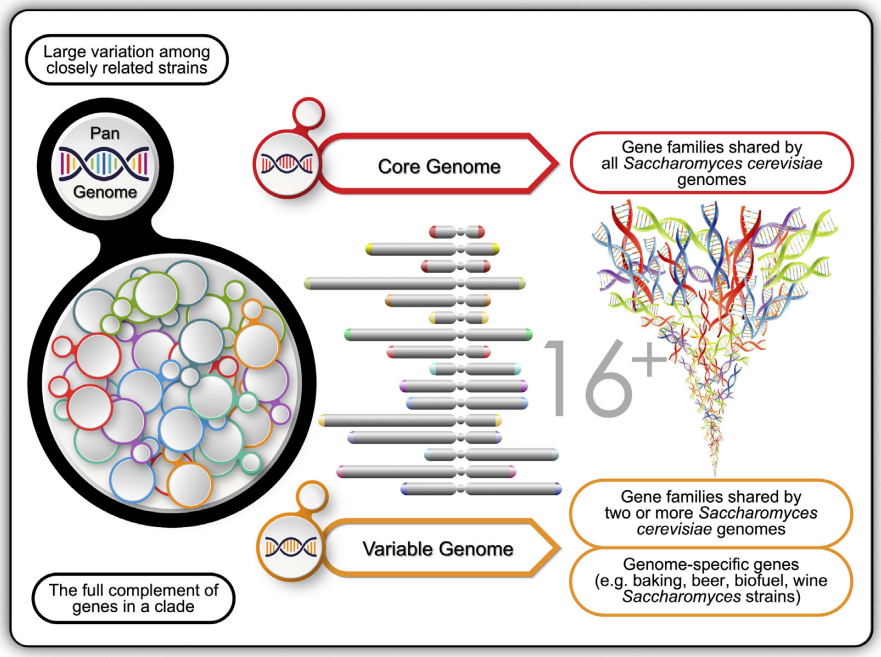

Figure 4. The construction of a synthetic *S. cerevisiae* pan-genome neochromosome. There is a diverse range of hundreds of *S. cerevisiae* strains with many displaying distinctive phenotypes. The *pan-genome* represents the entire set of genes within the *S. cerevisiae* clade, consisting of a *core genome*—containing genes shared among all strains within this species—and the *dispensable* or *variable genome*, which refers to genes and gene clusters found in two or more strains or to strain-specific genes and gene families.

a conceptual framework offers an interesting approach for deciphering key functionalities in microbial networks, their encoding genes, and their host organisms (Roume et al. 2015, Banerjee et al. 2018).

Given the impact of seasonal weather conditions and other environmental factors, it is less complicated to test the aforementioned conceptual framework in a more controlled model system, such as an uninoculated wine fermentation. A spontaneous wine fermentation represents an ephemeral microbial ecosystem, defined by a deterministic microbial succession where a keystone species, *S. cerevisiae*, will ultimately become the dominant species (Belda et al. 2021). Using fermenting grape must as a model ecological system has the advantage that fermentation conditions can be controlled and the keystone species is a well-studied model organism. Also, as *S. cerevisiae* is the dominant species capable of completing wine fermentation on its own, it is reasonable to assume that the vast majority of oenological keystone functions are encoded in its genome.

With the creation of a physiologically fit Sc2.0 strain nearing completion, it might be possible to move our research from a synthetic *S. cerevisiae* genome to the *de novo* synthesis of metagenomes that represent a whole yeast community in spontaneously fermenting grape must. Recently, the idea of encapsulating a representative synthetic metagenome in a single cell was proposed (Belda et al. 2021). This idea is not without risks, limitations, and challenges. However, this could enable researchers to reproduce and engineer microbial communities, combining the contributions of the different members of a microbial consortium in the genome of one of their keystone members (Fig. 5).

Despite being beyond the scale of existing technologies, it is still within the realm of possibility to design and construct a synthetic metagenome within the backbone of the Sc2.0 strain, representing key oenological functionalities of the main wine yeasts (e.g. fermentation performance, resilience, flavour activity, and antimicrobial activity; Belda et al. 2021). One can imagine a future *S. cerevisiae* wine strain carrying a synthetic metagenome that encodes all of the positive traits of other wine yeast species such as *Starmerella bacillaris* (*Candida zemplinina*), *Schwanniomyces vanrijiae* (*Debaryomyces vanriji*), *Hanseniaspora vineae*, *Lachancea thermotolerans*, *Metschnikowia pulcherrima*, *Pichia kluyveri*, and *Torulaspora delbrueckii* (Fig. 5). The technological challenges to be overcome in the construction of such a synthetic metagenome offers unique learning opportunities that could expand our understanding of keystone taxa as drivers of microbiome structure and functioning, how to use metabolic networks to resolve ecological properties of microbiomes in different terroirs (Roume et al. 2015, Banerjee et al. 2018, Belda et al. 2021, Conacher et al. 2021).

Synthetic yeast communities representing different terroirs

A parallel, complementary approach to the synthetic representation of a blend of desirable wine yeasts in a single *S. cerevisiae* cell is to construct a consortium of synthetic versions of those desirable yeast species (Fig. 6). This could stretch the realms of future possibilities from synthetic metagenomes to synthetic yeast communities and synthetic terroirs (Walker and Pretorius 2022). These seemingly utterly bewildering concepts are discussed here because synthetic biology is not just a nascent field of research that applies engineering principles to introduce 'gain-of-function' at higher-order scale (McCarthy and Ledesma-Amaro 2019). The vision of future wine yeast research is no longer constrained by a 'pure monoculture bias' as an endeavour to ensure consistent wine fermentation from vintage to vintage, and from terroir to terroir.

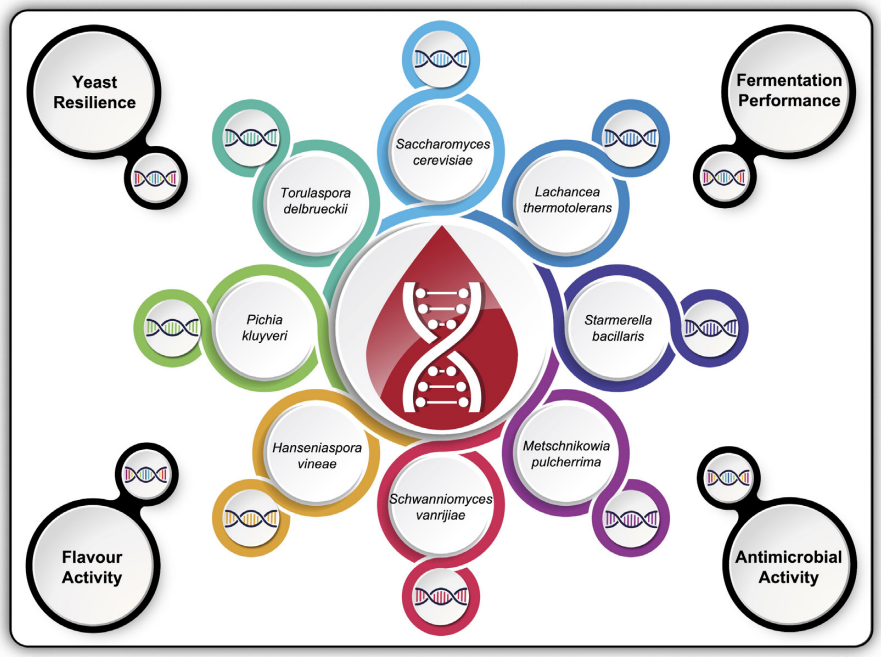

Figure 5. The idea of encapsulating a representative synthetic metagenome in a single cell. It is proposed to design and build a synthetic metagenome, which represents multiple grape-related yeast species, in a single *S. cerevisiae* cell. For example, a synthetic metagenome containing the genes from non-*Saccharomyces* yeast species could reinforce and/or complement the oenological traits of *S. cerevisiae* wine strains, such as resilience, fermentation performance, flavour production, and antimicrobial activity to curb spoilage. Conceptually, a wine strain of *S. cerevisiae* that encapsulates a representative synthetic metagenome could also uncover the complexity of multispecies interactions in wine ferments.

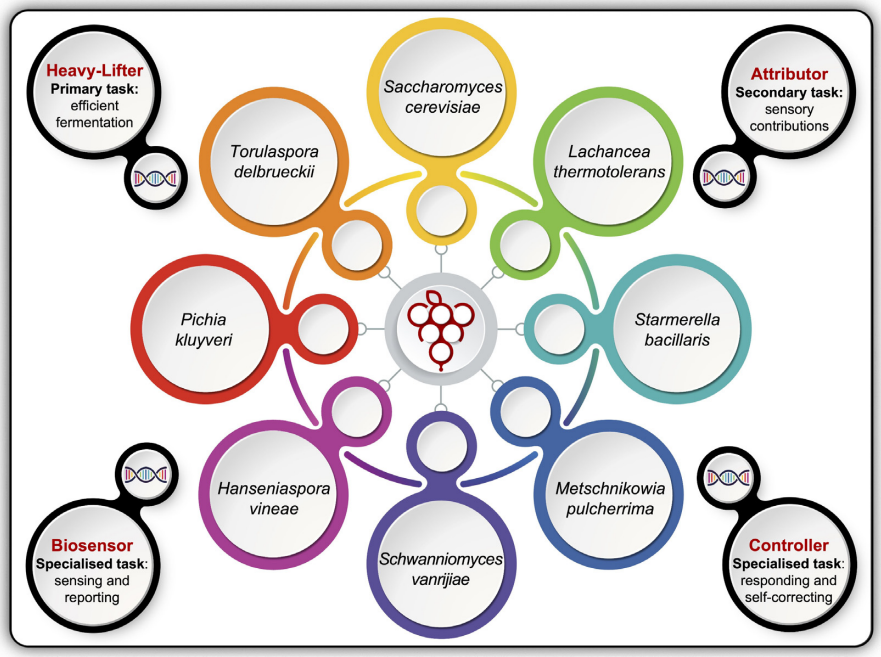

Figure 6. The idea of synthetic multiplexed yeast consortia with specialization of metabolic tasks. It is proposed to develop yeast consortia to carry out defined roles without the entirety of metabolic burden placed on an individual member. The primary role of the 'heavy-lifting' members is to catalyse the rapid, complete, and efficient conversion of grape sugars to ethanol, carbon dioxide, and other minor, but important metabolites without the development of off-flavours. The 'attributing' population is responsible for the secondary tasks, such as the biosynthesis of flavoursome compounds. The 'biosensor' cells specialize in sensing the conditions in the ongoing fermentation and relay information to the 'controller' cells to facilitate automated self-correction activities.

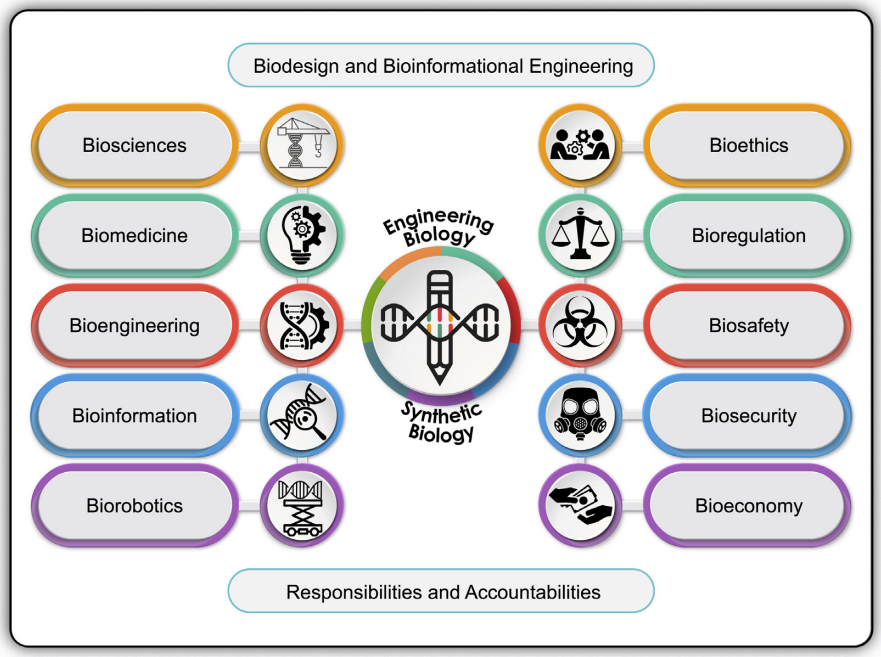

Figure 7. Guard rails are crucial to frontier science. The convergence of Engineering Biology (Synthetic Biology) and Bioinformational Engineering demands individual and collective responsibility and accountability from researchers. Safety and ethical standards must be embedded in transparent governance structures. The possession of specialist technical expertise entails a special obligation to provide information proactively and to take part in public debate about the uses to which innovations are put.

It is envisaged that a hallmark of a synthetic wine yeast consortium will be the specialization of metabolic tasks (Brenner et al. 2008, Tsoi et al. 2018). The division of labour amongst the members and compartmentalization of function will enable the synthetic yeast consortium to perform defined roles without the totality of burden be carried by an individual member. For example, while *S. cerevisiae* will do the 'heavy-lifting' in terms of the alcoholic fermentation (Goold et al. 2017), other synthetically customized members could be responsible for producing novel aromas (Swiegers et al. 2005, van Wyk et al. 2018), such as raspberry ketone (Lee et al. 2016) and β-ionone (Timmins et al. 2020), or conduct malolactic fermentation *in lieu* of *O. oeni* to reduce ethyl carbamate (Coulon et al. 2006) and bioamine formation (Husnik et al. 2006).

Other valuable additions to a synthetic multiplexed yeast consortium are whole-cell biosensors and controllers (Williams et al. 2016). The biosensor cells will sense fermentation conditions inside the winemaking vessel and transmit information from ongoing fermentation back to the winemaker so that adjustments can be made as needed (Dixon et al. 2021b). Alternatively, in self-regulating synthetic consortia, the biosensing yeast cells can relay information to controller cells (Walker and Pretorius 2022).

Biosensing cells can be engineered to detect and respond to target molecules with high sensitivity and high specificity (Williams et al. 2017, Carpenter et al. 2018). Examples of potential target molecules for biosensors include hydrogen sulfite (cabbage-like off-odour), volatile acids (vinegary off-favour), as well as volatile phenols and their glycosidic metabolites as biomarkers for smoke taint.

In a real-time, self-correcting *biosensor-controller* system, dynamic autonomous feedback-loops can be installed to prevent stuck fermentations and the production of off-flavours. For instance, fructose *biosensors* engineered to detect residual unfermented sugars can report that information to *controllers* so that they can induce hexose transporters in adjacent *heavy-lifters* for importation and fermentation of those residual sugars (Walker and Pretorius 2022). If nitrogen is depleted in such a self-regulating consortium, the consequential formation of unwanted hydrogen sulfite can be circumvented by informing an automated bioreactor device to add more diammonium phosphate to the fermenting grape must.

At this stage, these are just hypotheses that need to be developed, tested, and refined over the years and decades to come. However, they serve as a vision of how emerging trends and technologies in the development of engineered synthetic yeast communities could benefit wine fermentation performance through the sharing of capabilities, and eventually introducing new traits that are not possible through current practices (Walker and Pretorius 2022).

Beyond the frontiers

Frontier knowledge and exploration of audacious research ideas constitute the lifeblood of knowledge creation, scientific progress, technological innovation, economic development, and prosperity. This is especially true in today's highly contested world, which is ramping up the speed of technology development for geostrategic advantage and where technical expertise and know-how have already taking over so much of the global economy. Drawing on existing knowledge and data from seemingly unrelated fields of research, the generation of bold ideas is also the main driving force behind scientific progress and innovation in the wine industry.

In uncertain and challenging times like these, adversity can spark ingenious ideas and reveal opportunities that are often disguised as insoluble problems. Capturing and exploring those inventive ideas are also important to the harnessing of the confluence of biodesign and bioinformational engineering. High-throughput biofoundries offer a near-perfect platform where the

editing of wine yeast genomes can draw on the power of artificial intelligence, machine learning, automated robotics, quantum computing, and other biocyber technologies (Hillson et al. 2019). It is likely that the convergence of these emerging technologies will forge new research frontiers that could help future-proof winemaking against unexpected disruptions.

Crossing new frontiers by exploration of the concepts and ideas proposed in this article will present the wine industry and all of its stakeholders—researchers, practitioners, policymakers, regulators, commentators, and consumers—with some big questions about the potential benefits and risks of these emerging technologies (Sliva et al. 2015). The applications of frontier science and technology in winemaking are not shaped by science and scientists alone—acceptance depends on a complex interchange of regulatory, cultural, societal, economic, and political factors (Fig. 7). Therefore, the responsibility of forefront wine scientists does not end at the laboratory door. Pioneering researchers must always embrace the concept of individual and collective responsibility and accountability. Responsible trailblazers in emerging wine science understand that biosafety guard rails and ethical standards do not hinder their groundbreaking research but help pave the way to knowledge generation and value-adding innovation in a well-governed way.

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